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Archives of Internal Medicine

VOLUME 37 MARCH, 1926 NUMBER 3

EXPERIMENTAL RENAL INSUFFICIENCY

THE EFFECTS OF HIGH PROTEIN DIET IN THE PRESENCE OF LOW RENAL FUNCTION ON THE KIDNEYS, AORTA AND LIVER; CHANGES IN THE BLOOD PRESSURE AND CONCENTRATION OF BLOOD METABOLITES *

I. CONTROLS ON NORMAL DIET

HILDING ANDERSON, M.D.

DULUTH, MINN.

This investigation was undertaken to determine the effect of prolonged renal insufficiency on the blood pressure, and the influence of high or low protein diet on the course of chronic renal disease.

The real cause of the progressive development of renal insufficiency in chronic nephritis is not clear. One view is that it is due to repeated infections.[1] Day [2] holds that it is due to a specific infection. Bell [3] believes that each new infection occurring in a nephritic patient causes closure of more glomerular capillaries and leaves the patient with a smaller kidney filter than before. Carter, Howe and Mason [4] suggest as a possibility that progressive damage to the kidneys may result from repeated minimal injury by means of irritating food substances, some of which are known and others as yet unrecognized. Injury to the remaining functioning tissue by high concentration of retained metabolites or by overactivity are other possible causes of the progressive development of insufficiency.

The opinions of different writers vary somewhat concerning the injury to the kidney produced by high concentrations of nonprotein

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1. O'Hare, J. P.: Compatibility of Long Life with Low Kidney Function, J. A. M. A. **73**:248 (July 26) 1919.

2. Day, H. B., and Clarke, J. K.: An Experimental Study on the Origin of Idiopathic Nephritis, Lancet **2**:546 (Sept. 11) 1920.

3. Bell, E. T., and Hartzell, T. B.: Etiology and Development of Glomerulonephritis, Arch. Int. Med. **29**:768 (June) 1922.

4. Carter, H. S.; Howe, P. E., and Mason, H. H.: Nutrition and Clinical Dietetics, 1917, p. 393.

nitrogen in the blood. Some maintain that high concentrations are injurious.[5] Others tend toward the opposite view.[6]

Methylguanidin, a substance that may be produced from creatinin, is suggested by Boyd[7] and Myers[8] as a possible cause of uremic symptoms. Major's[9] recent work supports this view.

It is well known that a gradual rise of blood pressure is usually observed in chronic glomerulonephritis. This has been attributed by some observers to progressive atrophy of renal tissue.[10] The problem has been attacked experimentally by the removal or destruction of a large part of renal tissue. Pilcher[11] produced renal insufficiency by ligating branches of the renal arteries but obtained no cardiac hypertrophy even after a year. Paszler and Heinike[12] report a marked left ventricular hypertrophy in seven dogs after surgical reduction of kidney parenchyma. A rise in blood pressure in all seven also is recorded. The average increase was 21.5 mm. of mercury. The pressure was measured by means of a cannula in the femoral artery. These experiments have been widely quoted. Janeway[13] ligated branches of the renal arteries in nine dogs. Four of the dogs died. Three of those which died and two of those which survived showed a slight rise of blood pressure. The pressure was taken by means of a cuff on the foreleg.

Major[9] has recently reported some experiments which show that injections of various guanidin compounds raise the blood pressure in dogs. This is the same substance to which Myers[8] and Boyd[7] refer as a possible cause of the symptoms of uremia. Major's experiments were carried out under anesthesia with a cannula in the carotid artery.

Aside from the preliminary report of Bell, Clauson and Hartzell[14] no one has succeeded in producing in animals a disease comparable to

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5. Frothingham and Smillie, quoted by Carter, Howe, Mason (Footnote 4). Foster, N. B.: Uremia, J. A. M. A. **76**:281 (Jan. 29) 1921. Vaughn, V. C.: The Value and Limitations of a Salt-Free Diet and Restriction of Fluid in Nephritis, J. A. M. A. **53**:1789 (Nov. 27) 1909. Hewlett, A. W.; Gilbert, F. O., and Wickett, A. D.: Toxic Effects of Urea on Normal Individuals, Arch. Int. Med. **18**:636 (Nov.) 1916.

6. McLean, F. C.: Mechanism of Urea Retention in Nephritis, J. Exper. Med. **26**:181 (Aug.) 1917. McLean, Hugh: Treatment of Nephritis, Lancet **1**:407 (Feb. 28) 1924.

7. Boyd, F. D.: Nonprotein Nitrogen of the Blood, Especially in Its Relation to Nephritis and Renal Function, Edinburgh M. J. **16**:265 (April) 1916.

8. Myers, V. C.: Significance of Metabolism of Creatin and Creatinin with Special Reference to Nephritis, M. Rec. **91**:127, 1917.

9. Major, R. H.: Relationship Between Certain Products of Metabolism and Arterial Hypertension, J. A. M. A. **83**:81 (July 12) 1924.

10. Jawein, G.: Berl. klin. Wchnschr. **57**:869, 1920.

11. Pilcher: On the Excretion of Nitrogen Subsequent to the Ligation of Successive Branches of the Renal Arteries, J. Biol. Chem. **14**:389, 1913.

12. Paszler and Heinike: Verhandl. d. deutsch. Gesellsch. f. Chir. **9**:99, 1905.

13. Janeway, T. C.: Note on Blood Pressure Changes Following Reduction of Renal Arterial Circulation, Proc. Soc. Exper. Biol. & Med. **6**:109, 1908.

14. Bell, E. T.; Clawson, B. J., and Hartzell, T. B.: Experimental Glomerulonephritis, Am. J. Path. **1**:247-258 (May) 1925.

human chronic glomerulonephritis. Nephroses are readily produced by uranium, potassium chromate, chloroform and many other substances, but these conditions do not give a chronic renal insufficiency. The animals with acute renal insufficiency either die or recover within a short time. Even the acute glomerulonephritis produced by Pappenheimer, Hyman and Zieman [15] by means of bacteria injected directly into the renal artery is transitory.

For these reasons various workers have attempted to produce chronic renal insufficiency by removing or destroying a large part of the renal tissue.

Pilcher [11] and Janeway [13] ligated portions of the renal blood supply and studied the results. The method is not satisfactory because it is not possible to judge with sufficient accuracy the amount of tissue which is incapacitated and because a mass of toxic necrotic material remains.

Several workers have removed by operation various proportions of the total kidney tissue.[16] All agree that about three fourths may be removed without causing death. Karsner found a moderate retention of nitrogen bodies after removal of this amount, but only for a few days. Pearce found no change as evidenced by study of the urine.

METHODS

It was decided for the purposes of this study to remove a large proportion of the kidney substance surgically. When this is being done, the kidney cortex could be reduced any desired amount and a proportion found which would simulate the reduced filter found in chronic nephritis. It was realized that the two conditions would not be exactly similar; in chronic nephritis practically all of the glomeruli are at least partially closed,[17] whereas following partial nephrectomy, the small number which remain are entirely normal.

The means chosen for watching the course of the changes in the animals were periodic analysis of the blood for creatinin and urea nitrogen, determination of blood pressure, examination of fundus and of the urine, and observations of body weight.

When the increase of creatinin was definite, the determination of urea was not always made, especially in the later experiments.

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15. Pappenheimer, A. M.; Hyman, H. T., and Zeman, F. D.: Acute Glomerular Lesions Following Injections of Bacteria into the Renal Artery, Proc. New York Path. Soc. **16**:73, 1916.

16. Karsner, H. T.; Bunker, H. A., and Grabfield, G. P.: A Note on the Immediate Effects of Reduction of Kidney Substance, J. Exper. Med. **22**:544, 1915. Pearce, R. M.: The Influence of the Reduction of Kidney Substance upon Nitrogenous Metabolism, J. Exper. Med. **8**:632, 1908. Bradford, J. R.: Results Following Partial Nephrectomy and the Influence of the Kidney on Metabolism, J. Physiol. **23**:415, 1898.

17. Herxheimer: Ueber den jetzigen Stand unserer anatomischen Kentnisse der Nephritis und Nephropathien, München. med. Wchnschr. **65**:283, 1918.

Animals.—The animal used for these experiments was the rabbit. Only healthy animals were used. The blood and urine were analyzed and the blood pressure and weight determined previous to the second operation in each case.

Operative Procedures.—The operative procedures followed Bradford's technic rather closely. Two operations with certain exceptions [18] were performed on each animal. A wedge shaped piece was removed from the left kidney at the first operation. After ten days or two weeks the right kidney was removed in toto.

In the first eighteen animals operated on, the average weight of the portions removed from the left kidney was 3.07 Gm. Since all of these animals died of renal insufficiency sooner or later, a smaller portion averaging 2.08 Gm. was removed from the remainder. It is clear that some necrosis occurred in the kidney at the site of the incisions and that any tubules that were cut at any point would undergo atrophy. Therefore, the percentage by weight would indicate only approximately the amount of functioning cortex remaining. If one third of the left kidney were removed, it is obvious that after complete healing less than two thirds would be functioning.

The average weight of the kidneys removed in toto, usually the right, was 9 Gm.[19] The total weight of both kidneys was taken as twice the weight of the right in each case, and was probably only approximately correct. The percentage removed and the percentage remaining were calculated on this basis. The total amount removed varied between 62 and 68 per cent. The amount remaining varied between 32 and 38 per cent.[19] Five rabbits were operated on only once. The left kidney was removed in toto from three (Rabbits 34, 8 and 35) and in part from two (Rabbits 33 and 34).

Blood Analysis.—At irregular intervals of ten days or more 5 or 6 cc. of blood was drawn by means of a syringe from an ear vein.

The blood analysis was done according to the methods advocated by Folin and Wu.[20]

In the earlier work comprising about 35 per cent of the experiments all the blood analyses were personally made and most of the solutions were personally prepared by the author. Many of the analyses of the remaining experiments were made by Fred Rytz and Florence Madsen.[21]

18. In the case of diet experiments requiring removal of 15 per cent or 50 per cent of kidney substance, only one operation was performed.

19. Rabbit 15 not included.

20. Folin, Otto; and Wu, Hsien: A System of Blood Analysis, J. Biol. Chem. **38**:81 (May) 1919.

21. The urease method for urea determination using whole blood was employed by these two chemists.

Urine Examinations.—The urine was collected from the individual animals in a metabolism cage. The volume, specific gravity and presence or absence of albumin were determined and a microscopic examination made on each specimen. When determining the excursions of the variations in specific gravity, the urine was collected several times daily.

Fundus Examination.—The fundi were examined from time to time in thirty-two of the rabbits by means of a Welch-Allyn electric ophthalmoscope. Near the end of the study ten of the diet animals were carefully examined by means of a red-free light in order to detect minute blood vessel changes.[22]

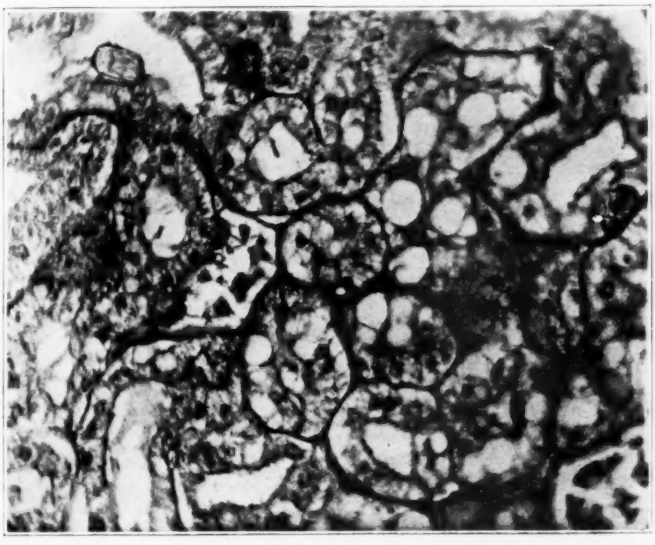

Fig. 1 (Experiment 24).—Kidney remnant forty-six days after removal of right kidney; severe hydropic changes.

Blood Pressure.—The blood pressure was taken in the central artery of the ear by means of an instrument devised for the purpose and described in 1923.[23]

This method is, I believe, superior to any other method which I have seen described. It does not cause any discomfort to the animal

22. Vogt, Alfred: Ueber eine vertikale Streifung, welche an der Vorderfläche der Netzhant jungerer Individuen im rotfrien Licht wahrgenommen wird, Klin. Monatsbl. d. Augenh. **60**:47, 1919.

23. Anderson, Hilding: Demonstration of an Instrument for Taking Repeated Blood Pressures in Rabbits, with Report of Some Experiments, Proc. Soc. Exper. Biol. & Med. **20**:295, 1923.

and may be repeated ad libitum over long periods of time. Using a cannula in an artery [12] requires an anesthetic and can be done only once. A cuff on the foreleg is not satisfactory because of the difficulty of palpating the small foot arteries.[13] Cutting out a portion of skin from the leg and holding the animal forcibly is very apt to cause marked variations in the pressure.[24] In the method used in this study, the variations obtained are only those incidental to the changes in diameter in a peripheral vessel. If heat radiation is carefully controlled, the variations may be largely controlled.

Diets.—In the main, only two diets were used, a normal mixed diet and a high protein diet.

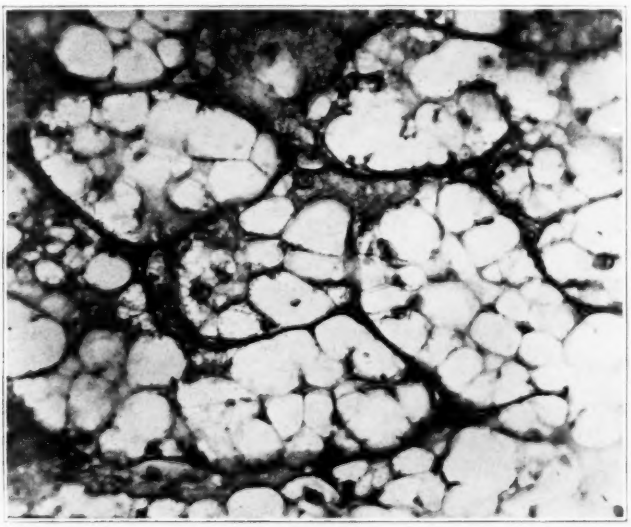

Fig. 2 (Experiment 25).—Kidney remnant one hundred five days after removal of right kidney; extreme hydropic degeneration.

The normal mixed diet included hay, white bread, carrots, cabbage, celery tops, lettuce, and fresh grass in season. Some of the animals also received oats.

The high protein diet consisted in a bread containing by weight between 55 and 60 per cent of beef.[25] The other ingredients were

24. Nuzum, F. R.; Osburn, Margaret; and Sansum, W. D.: The Experimental Production of Hypertension, Arch. Int. Med. **25**:492 (April) 1925.

25. This bread may be prepared as follows: The bone, fat and connective tissue are carefully removed from 15 pounds (6.8 Kg.) of roundsteak and the meat passed twice through a fine meat grinder; then 25 parts of meat, 16 parts of graham flour, and 4 parts of ground, dried alfalfa are mixed thoroughly, and 10 teaspoons of baking soda and 3 of salt and water are added to make a stiff dough. This is baked for an hour in a hot oven, or until the bread is moderately hard.

alfalfa and graham flour. The alfalfa and graham flour furnished roughage and vitamins. This bread contained about 30.5 per cent of protein, 19 per cent of fat, and 50.5 per cent of carbohydrate.

When on this diet each rabbit was given a single large leaf of lettuce once weekly.

The meat bread was eagerly consumed and the animals appeared to thrive on it.

A third diet consisting only of the whites of hard boiled eggs was used in a few instances. In the protocols this is referred to as "egg."

Controls.—Each step in the study was carefully controlled. The effects of the operative procedures were studied in thirty-seven animals on a normal diet before any were given high protein diet. The observations made on these thirty-seven included determinations of the effects on the weight, concentration of blood metabolites, and changes in the kidney remnant. All of the rabbits in the diet group were studied both before and after operation while still on a normal diet. The blood and urine were analyzed, the fundi examined, and the blood pressure and weight studied. Each thus became its own individual control. In addition, two normal rabbits not operated on (one male and one female from the same litter as some of the others in the diet group) have been included in the diet group and run as parallel experiments similar in every way except the operative procedure. A third normal control was started but it did not survive.

PROTOCOLS

The experiments on forty-four rabbits are included in this report. The rabbits are divided into three groups as follows: (1) those which died before or shortly after the second operation; (2) the operated controls; these survived the second operation but were not given a protein diet; (3) the diet group.

For the sake of clearness the results of blood analyses and blood pressure are given in table form or graph when more than a few determinations are recorded. The creatinin and urea nitrogen are given in milligrams per hundred cubic centimeters of blood and the blood pressure in millimeters of mercury.

GROUP 1

Rabbits 1 to 16 died either before or shortly after the second operation (removal of the right kidney). Most of the valuable data is grouped in Table 1. The blood pressure readings were within normal limits and are omitted.

Rabbits 5 to 16 all survived the removal of the wedge from the left kidney. At necropsy, two showed hydronephrosis of the remnant. In ten the kidney was healed and draining normally. Microscopic examination was made in eight. Five were normal except along the scar. One

showed moderate dilatation and two showed atrophy of the tubules. The atrophy in one of the last was marked and accompanied by scarring in the pelvis. The atrophy in the other was slight.

TABLE 1.—*Blood Analyses of Rabbits 1 to 16*

Rabbit	Creatinin	Urea Nitrogen	Rabbit	Creatinin	Urea Nitrogen
1	1.46	13.5	10	1.57	24.2
				1.9*	30.8*
2	1.11	24.0			
			11	1.5*	10.5*
3	1.47	20.0			
			12	1.6	....
4	1.6	11.1		1.97*	16.6*
5	1.3	27.0	13	1.8*	33.2*
	1.37				
	1.6	16.2	14	....	13.0
					12.6
6	1.03	30.0			27.5*
					22.0*
7	1.7	25.1	15	1.4	....
					30.3*
8	1.7	9.1			12.4*
	1.5*	9.8*		1.6*	14.0*
9	1.9*	18.0*	16	1.65*	....

* Rabbits from which a wedge of the left kidney had been removed; in all others both kidneys were intact.

TABLE 2.—*Data from Group 2, the Controls Operated On*

Rabbit	Days Survived*	Creatinin	Urea Nitrogen	Kidney Remnant
17	3	.....	.....	Atrophy; dilatation
18	4	3.3 (3)†	125.0 (3)	
19	6	.....	.....	Moderately hydropic
20	10	4.3 (5)	182.0 (5)	
21	13	4.7 (12)	293.0 (12)	Moderately hydropic
22	14	3.1 (3)	103.6 (3)	Atrophy; dilatation
		to	to	
		7.0 (14)	362.6 (14)	
23	21	3.1 (8)	44.8 (8)	Marked atrophy
24	46	2.4 (14)	23.5 (14)	Markedly hydropic
		to	to	
		4.0 (45)	150.0 (45)	
25	107	2.1 (6)	49.0 (6)	Extremely hydropic
		to	to	
		6.0 (106)	275.0 (106)	
26	117	1.7 (6)	49.0 (6)	Hydronephrosis
		to	to	
		5.1 (115)	216.4 (115)	
27	119	1.7 (14)	44.0 (14)	Marked atrophy
		to	to	
		3.8 (82)	156.0 (114)	
28	167	2.2 (66)	27.1 (48)	Atrophy; dilatation (calculus)

* The number of days the animal survived the second operation.
† The numbers in parentheses indicate the day following the second operation when the analysis was made (Figs. 1 to 5).

GROUP 2

This group includes those animals which survived both operations and were available for further study. This group serves as a series of operated controls for the diet group operated on (discussed in Part II of this article) and includes Rabbits 17 to 28. Table 2 contains the essential data.

NORMAL VALUES

Analyses were made on twenty-seven samples of normal blood taken from twenty-three rabbits not operated on eating a normal diet [26] and on twenty-eight samples taken from twenty-four rabbits that had been through the first operation (from 10 to 20 per cent of kidney substance removed).

The normal range of blood pressure was rather wide. Most readings lay between 70 and 86, although readings as low as 60 and as high at 90 were not uncommon. There was considerable variation between individuals. Usually any one individual yielded fairly consistent readings.

The specific gravity of the urine varied from 1.006 to 1.036 in the animals that were not operated on.

PROGRESS OF THE CONTROLS IN WHICH OPERATION WAS DONE

Rabbits 17 to 28 survived the second operation for a time varying from three to 167 days. None of these were given the high protein diet at any time but serve as a control series for the diet group.

Blood Metabolites.—The blood chemistry changes were characterized in general by a progressive accumulation of metabolites. There was a slight rise in the concentration of creatinin after the removal of the wedge from the left kidney. This was apparent only after averaging

TABLE 3.—*Analysis of Normal Blood*

	Number of Analyses	Average per 100 Cc.	High Figure	Low Figure
Creatinin	24	1.48 mg.	1.9 mg.	1.03 mg.
Urea nitrogen	20	17.79 mg.	30.0 mg.	9.1 mg.
Results of Analysis of Blood after First Operation				
Creatinin	23	1.67 mg.	2.1 mg.	1.3 mg.
Urea nitrogen	22	17.80 mg.	33.2 mg.	9.1 mg.

many cases. Beginning almost immediately after the removal of the right kidney there was a definite gradual increase in the concentration of creatinin and urea nitrogen which was progressive until death (Figs. 3, 4 and 5). Karsner [16] found no retention until more than three fourths had been removed. The rapidity of this increase in concentration bore an inverse ratio to the length of time the animals survived. Those in which the accumulation was slowed lived long, and those in which it was rapid died early.

All those which showed a progressive accumulation until death had apparently been deprived of too much kidney substance. Rabbits 17 to 22 lived fourteen days or less. The average weight of the wedge

26. Hammett, F. S.: Studies of Variations in the Chemical Composition of Human Blood, J. Biol. Chem. **41**:599 (April) 1920.

removed from the left kidney in these was 3.15 Gm. Rabbits 23 to 28 lived from twenty-one to 167 days. The average weight of the wedge was 2.12 Gm. The rate of accumulation was much slower in these.

The health of the animals continued apparently good until a decided retention had developed. When the concentration of urea rose as high as from 80 to 100 mg. and the creatinin to 3 or 3.5 mg., the animals began to lose weight and to become less lively. Weakness began to be apparent and the animals failed to eat well. Finally, as the concentration rose still higher a day or two before death, they quit eating entirely. The more severe symptoms usually developed only a few days before death. No convulsions were observed in any.

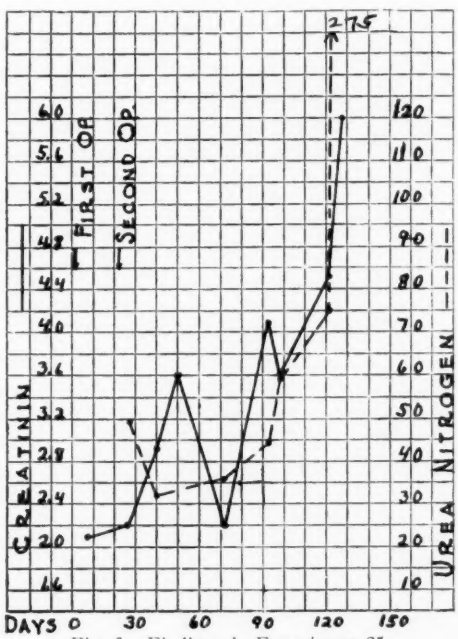

Fig. 3.—Findings in Experiment 25.

Pregnancy seemed to have an injurious effect on Rabbit 24 (Fig. 1). Two weeks after operation it showed only a small accumulation. About six weeks after operation it delivered young. Four days after delivery it died with a high urea, estimated at 400 mg.

Rabbits 26 and 28 showed a progressive rise in creatinin values and then a drop to normal. A second accumulation then began which progressed until death. At necropsy a calculus was found in each. These lived 117 and 167 days, respectively.

Rabbit 27 lived 119 days. The rise in creatinin was gradual but steady. There was no tendency toward a return to normal. At necropsy, the remnant showed signs of progressive closure of the tubules.

Urine Changes.—The urine changes were consistent with a simple reduction in the kidney filter.

The specific gravity tended strongly toward fixation (Table 4). This was greatest in those which suffered the highest degreees of insufficiency and least in those which were most nearly normal. Fixation occurred in most cases between 1.012 and 1.018. Rabbit 28 best illus-

TABLE 4.—*Specific Gravity of the Urine in Relation to the Operative Procedures*

Rabbit	Day of Experiment	Preoperative	After First Operation	After Second Operation
7	215 to 221	.....	1.008 to 1.020	.....
14	240 to 245	.....	1.016 to 1.022	.....
15	28 to 35	1.016 to 1.025	.....	.....
	185 to 189	.....	1.016 to 1.021	.....
16	8 to 11	1.018 to 1.032	.....	.....
	11 to 14	.....	1.028 to 1.033	.....
	64 to 67	.....	1.012 to 1.028	.....
27	64 to 68	.....	.....	1.011 to 1.017
	115 to 121	.....	.....	1.014 to 1.016
28	37 to 39	1.015 to 1.030	.....	.....
	52 to 57	.....	1.015 to 1.027	.....
	73 to 74	.....	1.025 to 1.031	.....
	74 to 80	.....	.....	1.017 to 1.024
	93 to 95	.....	.....	1.020 to 1.024
	119 to 123	.....	.....	1.015 to 1.018
	171 to 174	.....	.....	1.010 to 1.014
	176 to 178	.....	.....	1.008 to 1.014
36	212 to 216	.....	1.012 to 1.032	.....
	233 to 235	.....	.....	1.012 to 1.018
38	51 to 56	.....	.....	1.008 to 1.016
	143 to 149	.....	.....	1.012 to 1.020
	412 to 416	.....	.....	1.008 to 1.020
39	12 to 17	.....	1.010 to 1.030	.....
	25 to 28	.....	.....	1.016 to 1.018
42	31 to 33	.....	.....	1.013 to 1.020
43	26 to 28	.....	1.012 to 1.024	.....
	33 to 35	.....	.....	1.010 to 1.012
44	200 to 206	.....	1.012 to 1.026	.....
	258 to 262	.....	.....	1.022 to 1.031
	263 to 266	.....	.....	1.014 to 1.022
	436 to 440	.....	.....	1.006 to 1.020

trates the progression of fixation and loss of power to concentrate. Beginning with the concentration 1.031, it gradually lost concentration power until 1.014 was the upper limit.

The volume varied so greatly in both those operated on and those not operated on that no significant figures were obtained.

Albumin was found only occasionally and then only in traces unless complications had intervened.

Blood Pressure.—The blood pressure findings were quite unexpected. From clinical experience and from what little experimental

work had been done it was gathered that the blood pressure would surely rise proportionately to the reduction of the kidney substance.[27]

No hypertension resulted even in the cases of high grade insufficiency (Fig. 5).[28] The blood pressure was observed on thirty-three rabbits (including some which later fell into the diet group) over the period of time which they were under observation. No significant rise was observed in any as a result of or accompanying the reduction of kidney substance. Occasionally there seemed to be a slight postopera-

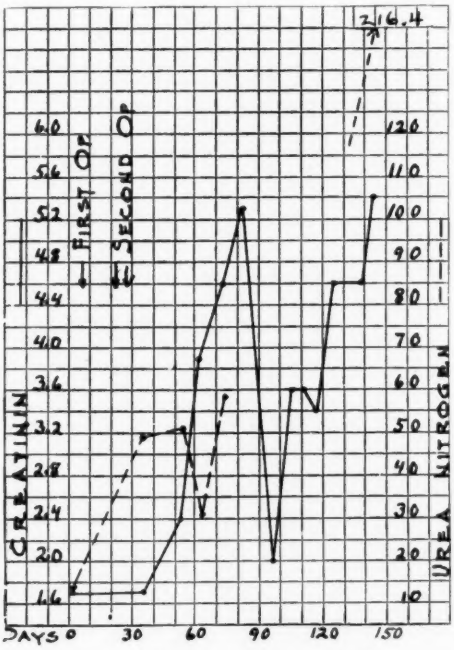

Fig. 4.—Findings in Experiment 26.

tive rise, as in Rabbits 15 and 16, but this was usually small and within normal limits and could often be accounted for by fear or an insufficient number of preoperative determinations. These rises were no greater than the variations frequently observed in normal animals. Moreover,

27. Jawein (Footnote 10). Heinike and Paszler (Footnote 12). Janeway (Footnote 13). Pilcher (Footnote 11). Cash, J. R.: Study of Blood Pressure Following Reduction of Renal Substance, Bull. Johns Hopkins Hosp. **35**:168 (June) 1924.

28. A preliminary report on some of these animals has been made by Anderson, Hilding: The Relation of Blood Pressure to the Amount of Renal Tissue, J. Exper. Med. **39**:707 (May) 1924.

just as great a postoperative fall was occasionally found (Rabbit 40, discussed in Part II of this article).

Of the thirty-three on which the blood pressure was observed the kidney substance had been reduced by approximately 65 per cent in twelve, and by from 12 to 50 per cent in eight. The remainder were normal at the time of the blood pressure determinations. Renal insufficiency obtained in most of the twelve and a number died as a result of insufficiency.

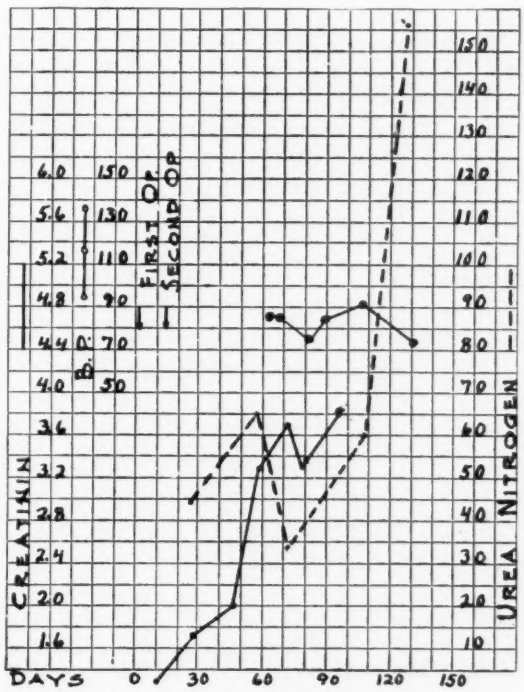

Fig. 5.—Findings in Experiment 27.

No renal hypertension was observed in any. Yet in special experiments with epinephrin, guanidin and intravenous saline increased blood pressure was obtained. This showed that the failure to obtain hypertension was not due to the method of determining the pressure.

Fundi.—The fundi were examined from time to time in a few of this group. A search was made in each case for signs of arteriosclerosis and albuminuric retinitis. No changes were observed. They all appeared essentially normal at all times.

Weight.—The weight maintained a normal course in each operated rabbit after recovery from the operations unless death intervened from some cause. A few days before death there was a fall in weight.

All of the rabbits were watched for convulsions and signs of edema. None were observed at any time. This is consistent with clinical findings. Patients may die from renal insufficiency and develop no edema. Many have no convulsions.[29]

NECROPSY FINDINGS IN CONTROLS THAT WERE OPERATED ON (TABLE 5)

Changes in the Kidney Remnant.—The gross change in the kidney remnant, unless complications were present, was a simple hypertrophy. If the left kidney had been removed or destroyed the right hypertrophied. This hypertrophy reached the high degree of 22.5 Gm. in the case of Rabbit 15 which lived 168 days after the destruction of the left kidney. The remnant of the left kidney, which had been operated on, after removal of the right hypertrophied to such an extent that it sometimes weighed as much or more than the right (Rabbits 26 and 27).

TABLE 5.—*Summary of Operative Procedures and Necropsies in Twelve Controls That Were Operated On*

Rabbit	Days Survived After Second Operation	Weight of Piece of Left Kidney Excised, Gm.	Weight of Right Kidney, Gm.	Percentage of Kidney Substance Remaining	Weight of Remnant of Left Kidney, Gm.	Microscopic Findings in Remnant
17	3	3.18	10.0	38.0	5.5	Atrophy; dilatation
18	4	3.32	10.0	33.4	...	
19	6	3.1	10.1	34.7	...	Moderately hydropic
20	10	3.7	10.0	31.5	7.2	Postmortem autolysis
21	13	3.0	8.4	32.1	4.2	Moderately hydropic
22	14	3.6	10.1	32.2	...	Atrophy; dilatation
23	21	1.96	8.5	38.5	5.6	Marked atrophy
24	46	1.9	7.5	37.3	7.0	Markedly hydropic
25	105	2.25	7.25	34.5	6.8	Extremely hydropic
26	116	3.0	10.4	35.7	10.3	Hydronephrosis (calculus)
27	119	1.9	7.9	37.3	9.7	Marked atrophy
28	168	1.8	7.9	38.6	7.0	Atrophy; dilatation (calculus)

Microscopically the remnant in all of this group showed marked pathologic changes. This was evidenced by varying degrees of hydropic change in the tubular epithelium or by atrophy of the tubules and glomeruli with or without dilatation.

The hydropic change was diffuse and differed in this respect from the small patches of hydropic degeneration found frequently in otherwise normal kidneys. In this group four showed hydropic change. These were Rabbits 19, 21, 24 and 25. The severity increased with length of life (Figs. 1 and 2), being most severe in Rabbit 25, which lived 107 days.

29. Foster (Footnote 5, second reference).

This hydropic change was not a result of the operation on the kidney. It did not occur in the animals operated on which showed no retention of metabolites or died before the second operation (Rabbits 7, 9, 14, 11, 12 and 33).

Two possibilities present themselves as the cause of this tubular injury: first, the toxic effect of retained urinary bodies; second, an exhaustion phenomenon following hyperactivity on the part of the renal epithelium, due to the greatly increased demand made on it.

In any case, this hydropic degeneration seems to represent the pathologic changes in the kidney remnant when the rabbits died of renal insufficiency uncomplicated by other local factors.

Complications resulted in some cases following the operation on the left kidney. Renal calculi occurred in four (Rabbits 26, 28, 39 and 40). Three of the four showed gross or microscopic signs of obstruction.

Obstruction occurred a number of times from other causes. In Rabbit 17 a necrotic mass of material was found in the pelvis. Rabbit 5 showed an old clot in the pelvis which extended into the ureter. In Rabbit 13 the pedicle had become twisted. All three showed gross or microscopic evidence of marked obstruction.

Rabbits 22, 23, 27 and 28 showed marked atrophy of all structures with varying degrees of tubular dilatation. They all gave the typical microscopic appearance of hydronephrosis but grossly there was no dilatation and the drainage appeared good. It was finally determined that removal of a portion of the single pyramid and progressive scarring about the excretory ducts brought about this condition (Rabbits 15 and 23).

Aorta.—No pathologic changes of any kind were observed in the aorta in any of the rabbits that had been fed a normal diet only. This shows that the changes observed in the high protein animals were not due to low renal function. Even those which died following a protracted renal insufficiency did not show arteriosclerosis.

SUMMARY OF CONTROLS IN WHICH OPERATION WAS PERFORMED

A condition of low renal function simulating the reduced kidney filter of chronic renal disease was produced in rabbits by removing portions of the kidneys by surgical means. Between 60 and 70 per cent of the total kidney substance was destroyed in this way.

Following the operative procedures in which over 60 per cent of kidney substance was destroyed, there was a retention of creatinin and urea nitrogen in the blood. This became progressively greater until death in Group 2.

There was no increase in blood pressure following the reduction of kidney substance, even in those which died from a slowly progressive renal insufficiency.

All in the second group died of renal insufficiency. The kidney remnant uniformly showed hypertrophy. Only four showed no gross or microscopic signs of obstruction. These four all showed diffuse hydropic change, which was probably due to overactivity.

The aorta appeared normal at necropsy. The liver in some exhibited fatty metamorphosis.

This article is in two parts; Part II will deal with partially nephrectomized rabbits on a high protein diet.

EXPERIMENTAL RENAL INSUFFICIENCY

THE EFFECTS OF HIGH PROTEIN DIET IN THE PRESENCE OF LOW RENAL FUNCTION ON THE KIDNEYS, AORTA AND LIVER; CHANGES IN THE BLOOD PRESSURE AND CONCENTRATION OF BLOOD METABOLITES*

II. Protein Diet Experiments

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It has long been held, apparently largely on theoretical considerations, that a chronic nephritis with nitrogen retention is benefited by a low protein diet. Hugh McLean [1] explains that the theory originated in a misconception of protein metabolism. He states (1924) that there is no proof that protein food is in any way injurious to the kidneys. He admits that a high concentration of nitrogen metabolites may be injurious and that in advanced cases of nephritis in which this condition is present it may be of value to limit the proteins.

Nevertheless, the therapy of protein limitation has found widespread acceptance in the treatment of Bright's disease and is extensively used.[2] Newburgh and Clarkson [3] report experimental studies in the feeding of a high protein diet to rabbits. They feel that they have produced a very definite renal injury by feeding a diet containing 36 per cent protein over a period of months. O'Hare,[4] on the other hand, seems to feel that repeated infections have more to do with injury to the kidneys than excessive protein in the diet.

A number of observers [5] have shown conclusivley that the concentration of nonprotein nitrogen in the blood of nephritic patients is increased

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1. McLean, Hugh: Treatment of Nephritis, Lancet **1**:407 (Feb. 23) 1924.

2. Janeway, T. C.: Management of Patients with Chronic Renal Disease, Am. J. M. Sc. **151**:157 (Feb.) 1916. Anders, J. M.: Treatment of Chronic Bright's Disease, M. Rec. **92**:311 (Aug. 25) 1917. Arnold, H. D.: Importance of Low Protein Diet in Chronic Nephritis, J. A. M. A. **55**:2193 (Dec. 24) 1910. Carter, H. S.; Howe, P. E., and Mason, H. H.: Nutrition and Clinical Dietetics, 1917, p. 393. Motzfeldt, K.: Dietetic Treatment of Renal Insufficiency, Acta med. Scandinav. **53**:611, 1920. Foster, N. B., and Davis, Helen B.: Effect of Water Intake on Nitrogen Retention in Nephritis, Am. J. M. Sc. **151**:49 (Jan.) 1916.

3. Newburgh, L. H., and Clarkson, S.: Renal Injury Produced in Rabbits by Diets Containing Meat, Arch. Int. Med. **32**:850 (Dec.) 1923.

4. O'Hare, J. P.: Compatibility of Long Life with Low Kidney Function, J. A. M. A. **73**:248 (July 26) 1919.

5. Chace, A. F., and Rose, A. P.: Dietetic Treatment of Nephritis, J. A. M. A. **69**:440 (Aug. 11) 1917. Folin, Otto; Denis, W., and Seymour, M.: The Nonprotein Nitrogen Constituents of the Blood in Chronic Vascular Nephritis as Influenced by the Level of Protein Metabolism, Arch. Int. Med. **13**:224 (Feb.) 1914. McLean, F. C.: Mechanism of Urea Retention in Nephritis, J. Exper. Med. **26**:181 (Aug.) 1917. Hewlett, A. W.; Gilbert, Q. O., and Wickett, A. D.: Toxic Effects of Urea on Normal Individuals, Arch. Int. Med. **18**:636 (Nov.) 1916.

or diminished markedly by corresponding changes in the quantity of protein in the diet. But it does not necessarily follow that these changes in the blood influence the general condition of the patient or the course of the disease. McLean [6] quotes data from a case of nephritis in which the blood urea was made to vary from 26 to 254 mg. per hundred cubic centimeters without changing either the subjective or the objective symptoms of the patient. Folin, Denis and Seymour,[7] on the other hand, noted headache, nausea, lassitude and gastro-intestinal disturbances in patients to whom they fed a high protein diet. They did not note any extensive increase in the blood urea. Opie and Alford [8] found experimentally that potassium chromate and uranium nitrate were more potent in producing nephritis in animals that were fed on a meat diet.

GROUP 3

The sixteen rabbits in Group 3 were fed a high protein diet (30.5 per cent protein) over varying periods of time. Thirteen were partially nephrectomized. They all recovered and returned to normal values of blood creatinin and urea before receiving the high protein diet.

They were divided into four subgroups. Subgroup A includes three normal rabbits. Subgroups B, C and D had been deprived of 12 per cent, 50 per cent and 65 per cent of kidney substance, respectively. Subgroups B and C include two rabbits each and Subgroup D includes nine.

PROTOCOLS

For the sake of clearness, the data in the protocols pertaining to the following points is given in table form or chart if many observations are recorded; namely, diet, creatinin and urea nitrogen concentrations, blood pressure and weight. In the diet column, the word protein is used to indicate the time when the animal was placed on the high protein diet, and the word normal to indicate the time when it was replaced on a normal diet.

The necropsies were made complete as a routine matter and included examination of the following: peritoneal cavity, amount of fat present, pleural cavities, lungs, heart, spleen, liver, stomach and intestines, kidneys, suprarenals, aorta and, in the female, tubes, ovaries and uterus. A microscopic examination was made of the lungs, heart, spleen, liver, intestine, kidneys, suprarenal, aorta, voluntary muscle and, in the female, of the tubes, ovaries and uterus. Only the positive or significant findings are given.

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6. McLean (Footnote 5, third reference).

7. Folin, Denis and Seymour (Footnote 5, second reference).

8. Opie, E. L., and Alford, L. B.: Influence of Diet upon Necrosis Caused by Hepatic and Renal Poisons, J. Exper. Med. **21**:1, 1915.

Subgroup A: Controls Not Operated On.—Two of these are shown in Figures 1 and 2. There was no increase of creatinin or urea nitrogen. The blood pressure remained normal. The animals ate the meat bread well and increased in weight. In Rabbit 30 there was moderate sclerosis of the aorta and a marked hydropic change in the liver. The kidneys were enlarged, together weighing 23 Gm. In Rabbit 31 there was a marked sclerosis of the aorta with extensive atheroma and calcification and saccular dilatation (Fig. 3). The kidneys together weighed 28.4 Gm. There was a marked hydropic change in the liver.

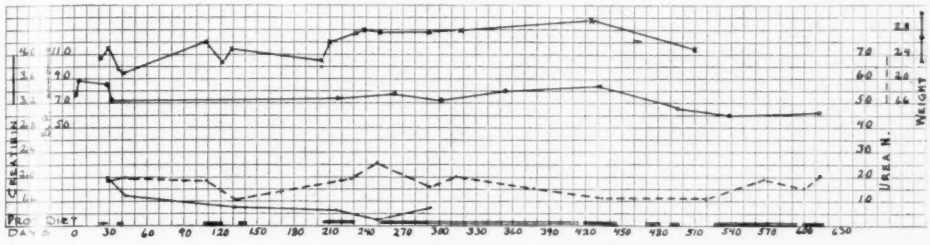

Fig. 1.—Findings in Experiment 30.

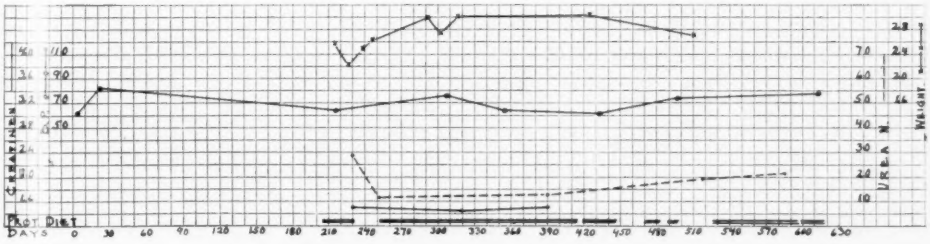

Fig. 2.—Findings in Experiment 31.

Subgroup B.—The kidney substance in these two rabbits (Rabbits 32 and 33) was reduced about 12 per cent. Each was subsequently kept on the protein diet fifty-two days. The creatinin did not increase, but there was a slight increase of urea nitrogen (35 mg.) in Rabbit 32. The blood pressure remained normal. The aorta of Rabbit 33 was normal, but there was moderate atheroma in Rabbit 32. Marked hydropic changes in the liver were noted in both animals.

Subgroup C.—These two animals (Rabbits 34 and 35) were deprived of about 50 per cent of the renal tissue by removal of the entire left kidney. Each was subsequently kept on the protein diet eighty-seven days. When killed at the close of the experiment both

were very fat. There were no changes in the retinal vessels. There was definite hypertrophy of the remaining kidney (14.7 and 12.3 Gm.). A slight atheroma of the aorta was found in Rabbit 34 and moderate atheroma in Rabbit 35. The liver showed moderate hydropic change in Rabbit 34 and a fatty metamorphosis in Rabbit 35. The blood pressure remained normal. A moderate rise in creatinin and urea nitrogen occurred (Table 1).

Subgroup D.—This group includes those rabbits which were deprived of 65 per cent of kidney substance and were fed a high protein diet.

TABLE 1.—*Analysis of Rabbit 35*

Day of Experiment	Diet	Creatinin	Urea Nitrogen
1 (first operation)	.....	...	....
6	.....	...	....
7	.....	1.8	17.5
22	Protein	...	....
35	.....	2.5	40.6
63	.....	1.7	35.2
92	Normal	...	....
95	Protein	...	....
96	.....	1.7	43.4
109	.....	2.4	37.1
113	Normal	...	....

TABLE 2.—*Analysis of Rabbit 36*

Day of Experiment	Diet	Creatinin	Urea Nitrogen	Blood Pressure	Weight, Kg.
1	.....	...	....	75	....
35	.....	...	...	84	....
193 (first operation)	.....	...	....	..	....
214	.....	2.0	....	..	....
218	.....	...	20.1	..	....
219	.....	...	....	78	....
228 (second operation)	.....	...	....	..	....
249	.....	...	...	90	....
254	.....	...	26.1	..	....
273	.....	1.8	....	..	....
280	.....	...	....	87	....
281	Egg	...	....	..	....
283	.....	..	...	..	2.27
285	.....	...	....	..	1.93
286	Normal	...	27.1	..	....

EXPERIMENT 36 (Female, Table 2).—The experiments were begun July 5, 1922; death occurred April 23, 1923, the two hundred and ninety-second day.

Day 1, the animal was three-fourths grown.

Day 193, the left kidney was removed; the weight was 7.8 Gm.; microscopically, it was normal.

Days 212 to 216, the specific gravity of eight specimens varied from 1.012 to 1.032.

Day 216, albumin was absent.

Day 228, the right kidney was operated on and between one third and one half of the cortex infarcted.

Days 231 to 235, the specific gravity in three specimens was from 1.012 to 1.018.

Day 234, albumin was absent.

Day 281, the animal was placed on an egg and bean diet.

Day 286, the animal was taken off the egg and bean diet; she had eaten practically nothing during the five days.

Day 290, the animal delivered young.

Day 292, the animal died. The total number of days on the high protein diet was five; she survived the operation on the right kidney sixty-four days.

At necropsy the right kidney weighed 11.9 Gm. Three large infarcts were found in the cortex. These areas were atrophied. The living cortex was rather soft; the ureter and pelvis appeared normal. There were no signs of obstruction.

Microscopic examination of the right kidney showed nothing abnormal in the functioning cortex. The infarcted areas were completely atrophied.

Hypertrophy of the right kidney was the abnormal finding.

EXPERIMENT 37 (Table 3).—Experiments were begun May 5, 1923; death occurred Nov. 15, 1923, the one hundred and ninety-fourth day.

Day 1, three areas totaling 50 per cent of the left kidney cortex were infarcted.

Day 34, the right kidney was removed; the weight was 8.2 Gm.; microscopically, it was normal.

Day 130, the creatinin was 1.7 mg.

Day 194, the animal died, having survived the removal of the right kidney 160 days. The total number of days on a high protein diet was twenty-three. The carcass was lost.

TABLE 3.—*Analysis of Rabbit 37*

Day of Experiment	Diet	Urea Nitrogen	Blood Pressure	Weight, Kg.
1 (first operation)	.....	.....	..	....
5	.....	10.75	..	....
34 (second operation)	.....	.....	..	....
40	.....	.....	88	....
90	.....	.....	70	....
105	Protein	.....	..	....
107	.....	.....	..	2.04
113	.....	.....	..	2.10
117	.....	36.15	..	....
119	Normal	.....	..	....
130	.....	21.7	..	....
133	Protein	.....	..	....
140	Normal	.....	..	....
151	.....	.....	71	....

EXPERIMENT 38 (Male, Fig. 4).—The experiments were begun March 18, 1922; death occurred July 1, 1923, the four hundred and sixty-ninth day.

Day 5, 2 Gm. of the left kidney was removed; microscopically, this was normal.

Day 20, the right kidney was removed; the weight was 7.3 Gm.; microscopically, it was normal.

Days 49, 50 and 52, albumin was absent (Table 4, Paper I).

Day 60, the fundi were normal.

Day 142, the fundi were normal.

Days 142, 143 and 146, albumin was absent.

Day 268, the fundi were negative.

Day 401, phenolsulphonephthalein elimination was 60 per cent.

Days 411 to 414, the animal was on egg diet.

Day 411, albumin was absent.

Day 414, a trace of albumin was present. The sediment showed occasional hyaline casts. The animal ate practically none of the egg offered it during these days.

Day 414, the animal was given a diet of meat bread (see methods); it ate small amounts of this.

Day 415, no urine was excreted in twenty-four hours.

Day 416, the first bowel movement was passed since the animal was placed on the egg diet on Day 411. The urine measured 147 cc.; specific gravity was 1.025; there was a trace of albumin and a few casts were present.

Day 417, the animal was replaced on a normal diet. The animal had eaten almost nothing since Day 410.

Day 430, phenolsulphonephthalein elimination was 45 per cent.

Day 469, the animal died, 449 days after the removal of the right kidney. The total number of days on the righ protein diet was seventeen.

EXPERIMENT 39 (Male, Fig. 5).—The experiments were begun May 3, 1923; death occurred March 15, 1924, the three hundred and eighteenth day.

Day 1, the animal was about three months old and not fully grown.

Day 3, the left kidney was operated on; 50 per cent of the cortex was infarcted.

Days 13 and 14, albumin was absent, but on Day 17 after the operation a trace was found (Table 4, Paper I).

Day 17, the right kidney was removed; the weight was 11.7 Gm.; microscopically, it was normal.

Day 271, the specific gravity of the urine was 1.015; albumin was +; the sediment showed occasional pus cells.

Day 272, the specific gravity of the urine was 1.025; albumin was absent.

Day 303, the right forefoot was lame; he refused to bear weight on it.

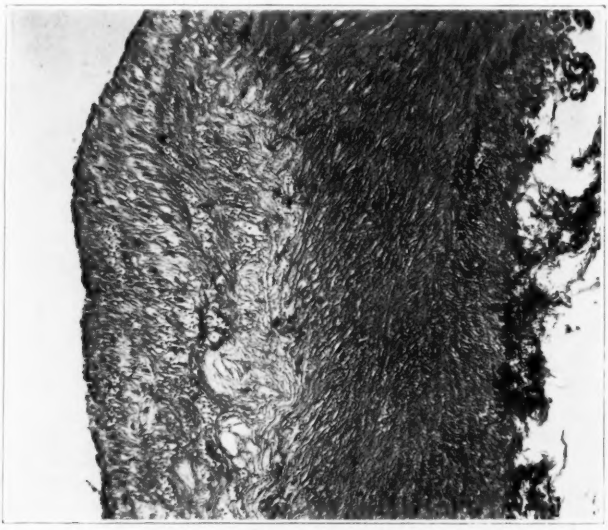

Fig. 3.—(Experiment 31).—Aorta of control not operated on after 324 days on high protein diet; marked atheromatous change in intima.

Day 318, the animal was sick and emaciated; the fur was soiled, and the left eye was suppurating in an old injury to the lid. Calcium totaled 12 mg. per hundred cubic millimeters of blood. He was killed by a blow on the head, 301 days after the removal of the right kidney. The total number of days on the high protein diet was 128.

At necropsy the left kidney weighed 11.5 Gm. The cortex was swollen, cloudy and wet, and there was a stone in the pelvis. The aortic ring was hard and calcified, otherwise apparently normal. The entire aorta was dilated and markedly sclerosed throughout its length. The walls were calcified and so stiff that they fractured when handled. There were many transverse, yellow rings and a few yellow plaques present. There was no marked thickening of the intima.

Microscopically the glomeruli and tubules of the kidney appeared hypertrophied; there were a few small areas of hydropic change.

Marked arteriosclerosis, renal calculus and renal hypertrophy were the abnormal findings.

EXPERIMENT 40 (Female, Fig. 6).—The experiments were begun Sept. 21, 1923; death occurred Sept. 11, 1924, the three hundred and fifty-seventh day

Day 2, 2.3 Gm. of the left kidney was removed; microscopically, this was normal.

Day 9, the right kidney was removed; the weight was 8.3 Gm.; microscopically, it was normal.

Day 107, the animal was pregnant.

Day 126, the animal was pregnant.

Day 128, she delivered young. The young died quickly.

Day 143, albumin was absent.

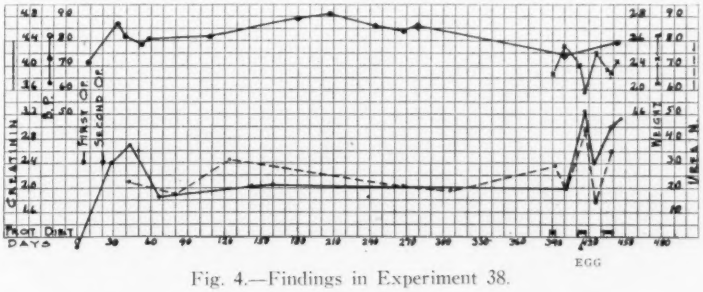

Fig. 4.—Findings in Experiment 38.

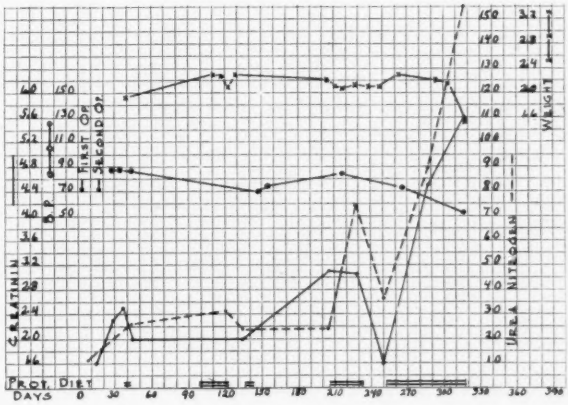

Fig. 5.—Findings in Experiment 39.

Day 179, calcium was 15.5 mg. per hundred cubic centimeters of blood.

Day 180, the specific gravity of the urine was 1.025; albumin was +; many red blood cells were present in the urine.

Day 331, the animal delivered young. She was placed in an individual cage and the young were carefully guarded, but they all died quickly.

Day 356, she appeared sick and refused to eat.

Day 357, she was sick and thin. At 9:15 p. m. she was given 0.1 Gm. of methyl guanidine sulphate intravenously; at 11 p. m. she died in mild convulsions. The animal survived the removal of the right kidney 348 days. The total number of days on the high protein diet was 238.

At necropsy the left kidney was found to be adherent by scar tissue to the abdominal wall; the cut surface was moist with yellowish striations; the pelvis was somewhat dilated; a stone was found in the beginning of the ureter.

The aortic leaflets of the heart were thickened and the ring was thickened and hardened; otherwise the heart was normal. The aorta contained many atheromatous plaques throughout its entire length.

Microscopically, there were occasional glomeruli in the kidney with fairly normal tubules but the great majority had disappeared entirely. The liver showed no degeneration.

The abnormal findings were renal calculus and pyelonephritis.

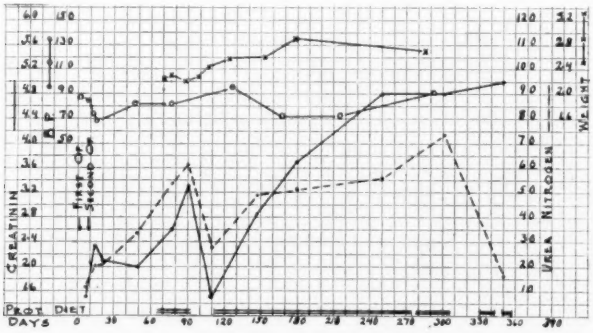

Fig. 6.—Findings in Experiment 40.

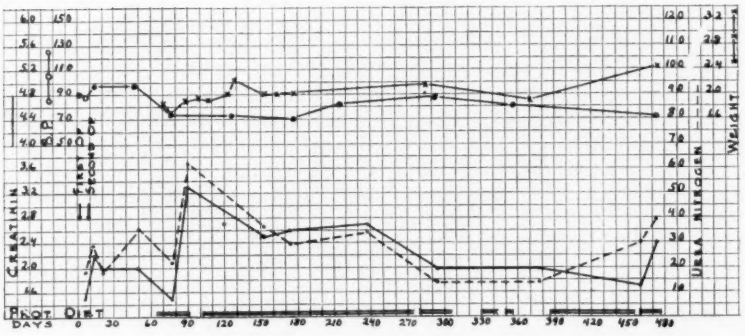

Fig. 7.—Findings in Experiment 41.

EXPERIMENT 41 (Male, Fig. 7).—Experiments were begun Aug. 21, 1923; death occurred Jan. 10, 1925, the four hundred and seventy-eighth day.

Day 2, 2.55 Gm. of the left kidney was removed; microscopically, this was normal except for occasional groups of lymphocytes.

Day 9, the right kidney was removed; the weight was 8.05 Gm.; microscopically, this was normal except for occasional groups of lymphocytes.

Day 14, urine examination of a twelve hour specimen showed a volume of 120 cc., with specific gravity 1.012.

Day 16, a twelve hour specimen showed a volume of 275 cc., with specific gravity 1.015.

Day 85, a twenty-four hour specimen showed a volume of 335 cc., with specific gravity 1.024 and a slight trace of albumin.

Day 182, the specific gravity of the urine was 1.025; albumin was absent.

Day 299, a twenty-four hour urine specimen had a volume of 171 cc., with specific gravity 1.020 and albumin + +.

Day 426, the fundi were normal.

Day 437, a twenty-four hour urine specimen had a volume of 78 cc., with specific gravity 1.034 and albumin absent.

Day 457, the fundi were examined by means of a red-free light. Nothing abnormal could be detected.

Day 478, the animal was killed by a blow on the head, 469 days after the removal of the right kidney. The total number of days on a high protein diet was 325.

At necropsy the right kidney was absent. The left weighed 10.2 Gm.; the operative wound was healed; the remainder appeared normal. The aorta was normal throughout its length. The heart, which was normal, weighed 6 Gm. There was much omental, mesenteric and perirenal fat.

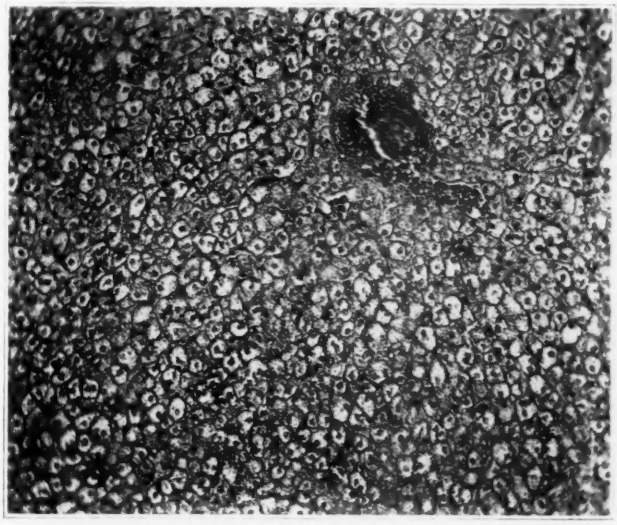

Fig. 8 (Experiment 41).—Hydropic change in liver 469 days after removal of 65 per cent of the kidney substance and after 325 days on high protein diet.

Microscopically, in the left kidney remnant there were a few lymphocytes about the operative scar, a few small areas of spontaneous nephritis and hypertrophy of the glomeruli and tubules. In the aorta there were degenerative changes in the media, with beginning calcification. In the liver there were hydropic change in the cells throughout the lobules and slight periportal infiltration of the lymphocytes (Fig. 8).

The abnormal findings were degenerative changes in the media of the aorta and hydropic change in the liver.

EXPERIMENT 42 (Male, Fig. 9).—The experiments were begun Aug. 10, 1923; death occurred Jan. 10, 1925, the five hundred eighteenth day.

Day 2, 50 per cent of the left kidney cortex was infarcted.

Day 30, a twenty-two hour urine specimen had a volume of 160 cc., with specific gravity 1.020; albumin was absent. The right kidney was removed; microscopically, it was normal except for occasional groups of lymphocytes.

Day 32, the urine specimen had a volume of 100 cc., with specific gravity 1.013.

Day 33, a twenty-four hour urine specimen had a volume of 250 cc., with specific gravity 1.020.

Day 184, the specific gravity of the urine was 1.027; albumin was absent.

Day 221, calcium in the blood was 16 mg. per hundred cubic centimeters.

Day 234, the specific gravity of the urine was 1.036; albumin was + +.

Day 308, a twenty-four hour urine specimen had a volume of 65 cc., with specific gravity 1.031 and albumin +.

Day 466, the fundi showed no signs of arteriosclerosis or albuminuric retinitis.

Day 484, a twenty-four hour urine specimen had a volume of 88 cc., with specific gravity 1.036; albumin was absent.

Day 497, the fundi were examined by a red-free light but nothing abnormal was found.

Day 518, the animal was killed by a blow on the head, 488 days after the removal of the right kidney. The total number of days on a high protein diet was 325.

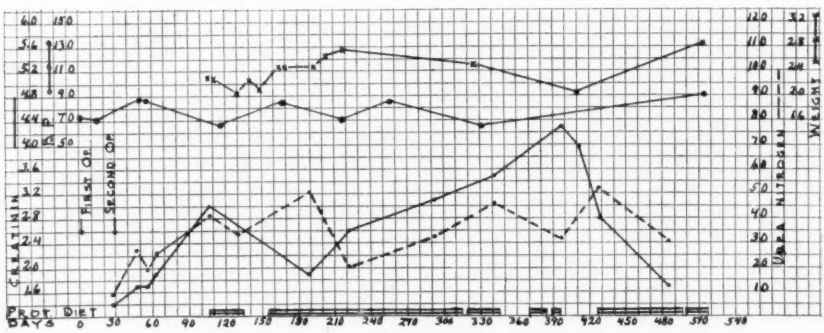

Fig. 9.—Findings in Experiment 42.

At necropsy the right kidney was absent. The left was bound down by adhesions; it weighed 9.1 Gm. The infarcted areas were healed and one was completely calcified; the noninfarcted areas appeared normal. The aorta was normal except for slight thickening about the mesenteric vessels.

Microscopic examination of the kidney remnant showed small areas of spontaneous nephritis; otherwise it was normal. The aorta was normal. The liver showed marked hydropic change in the cells, which was greatest at the centers of the lobules.

The abnormal findings were infarcts of the left kidney, one calcified, and hydropic change in the liver.

Experiment 43 (Female, Fig. 10).—The experiments were begun Aug. 10, 1923; death occurred Jan. 10, 1925, the five hundred twentieth day.

Day 1, the animal was pregnant, and not yet fully grown.

Day 2, 50 per cent of the left kidney cortex was infarcted.

Day 27, a twenty-three hour urine specimen had a volume of 465 cc., with specific gravity 1.012; albumin was absent.

Day 28, a twenty-five hour specimen had a volume of 245 cc., with specific gravity 1.024 (Table 4, Paper I).

Day 30, the right kidney was removed; the weight was 8.3 Gm.; microscopically, it was normal.

Day 34, a twenty-four hour specimen had a volume of 385 cc., with specific gravity 1.010.

Day 35, a twenty-four hour specimen had a volume of 400 cc., with specific gravity 1.012; albumin was absent.

Day 183, the specific gravity of the urine was 1.025; albumin was absent.

Day 226, the specific gravity of the urine was 1.026; albumin was absent.

Day 322, the animal delivered young, which were dead when found.

Day 341, a twenty-four hour urine specimen had a volume of 195, with specific gravity 1.019; albumin was + +.

Day 376, the animal delivered young. Three of this litter survived. These were the only young to survive of all those delivered during the year 1924.

Day 399, the animal delivered young, which died the same day.

Day 468, the fundi were normal.

Day 480, a twenty-four hour urine specimen had a volume of 198 cc., with specific gravity 1.021 and albumin + +.

Day 499, the fundi were examined with a red-free light; the vessels all appeared normal.

Day 515, the animal was pregnant.

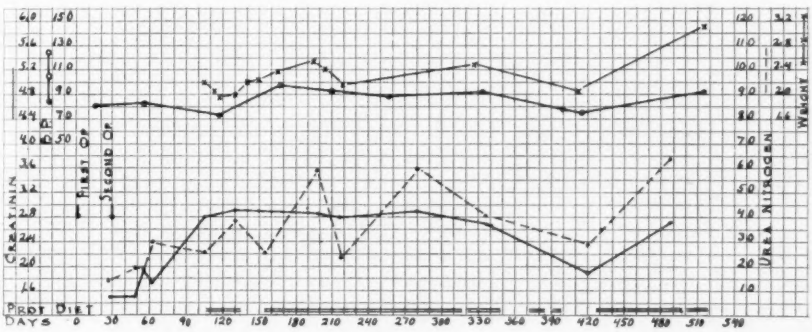

Fig. 10.—Findings in Experiment 43.

Day 520, the animal was killed by a blow on the head, 490 days after the removal of the right kidney. The total number of days on a high protein diet was 325.

At necropsy the right kidney was absent. The left was surrounded by large quantities of fat; the weight was 15.7 Gm.; the capsule was adherent over the operative infarcts; the infarcted areas were calcified. In the aorta there were a moderate number of atheromatous streaks and patches, and moderate sclerosis in the root. The heart weighed 10.5 Gm.; the pulmonary and aortic valves were both moderately thickened; the mammary glands contained milk.

Microscopically, the left kidney infarcted areas showed complete atrophy; there were scars from the spontaneous nephritis; otherwise it was normal. In the aorta there were marked atheromatous changes in the intima and beginning calcification in the media (Fig. 11). The liver showed marked hydropic change.

The abnormal findings were calcified infarcts in the left kidney, moderate atherosclerosis in the aorta and hydropic change in the liver.

Experment 44 (Female, Fig. 12).—The experiments were begun Aug. 8, 1922; death occurred Jan. 10, 1925, the eight hundred eighty-seventh day (Table 4, Paper I).

Day 158, 2.35 Gm. of the left kidney was excised; microscopically, this was normal (Fig. 13).

Day 165, the animal delivered young.

Day 206, the right kidney was removed; the weight was 8.35 Gm.; microscopically, it was normal.

Day 246, the animal was placed on an egg and bean diet.

Day 250, she looked ill and was replaced on a normal diet. She had apparently eaten little of the beans and eggs.

Day 258, the animal looked extremely sick and had rigors.

Day 258, albumin was +; there were many hyaline casts and occasional red blood cells in the sediment. The diagnosis was pyelonephritis.

Day 261, albumin was absent; the sediment contained a moderate number of hyaline casts and pus cells. Methenamine was administered in the drinking water.

Day 264, albumin was absent; the sediment contained a moderate number of hyaline casts and pus cells. Methenamine was administered in the drinking water.

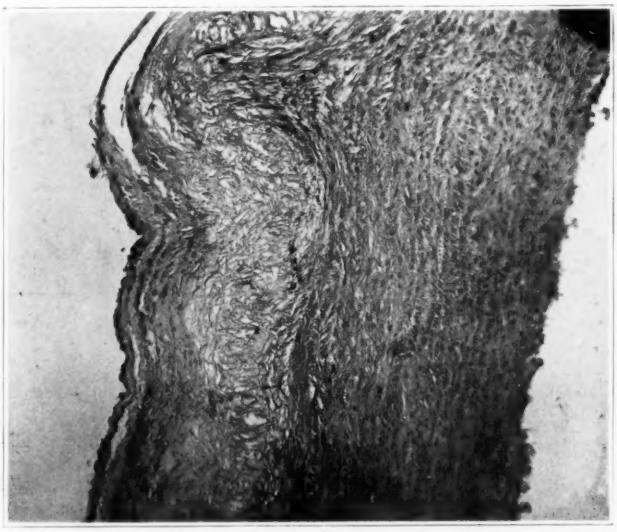

Fig. 11 (Experiment 43).—Aorta 490 days after removal of right kidney and after 324 days on high protein diet; marked atheromatous change in the intima; 65 per cent of kidney substance removed.

Day 266, albumin was absent; casts and pus were absent. The animal appeared much better and was replaced in the cage with the other rabbits.

Day 271, she appeared thin but lively. The fundi showed nothing abnormal.

Day 275, the urine specimen had a volume of 56 cc.; albumin was absent; specific gravity was 1.035.

Day 279, the urine specimen had a volume of 310 cc., with specific gravity 1.010.

Day 284, the animal was still very thin, and was pregnant.

Day 439, she delivered young.

Day 539, in the urine specimen albumin was +; specific gravity was 1.012; there were many pus cells in the sediment.

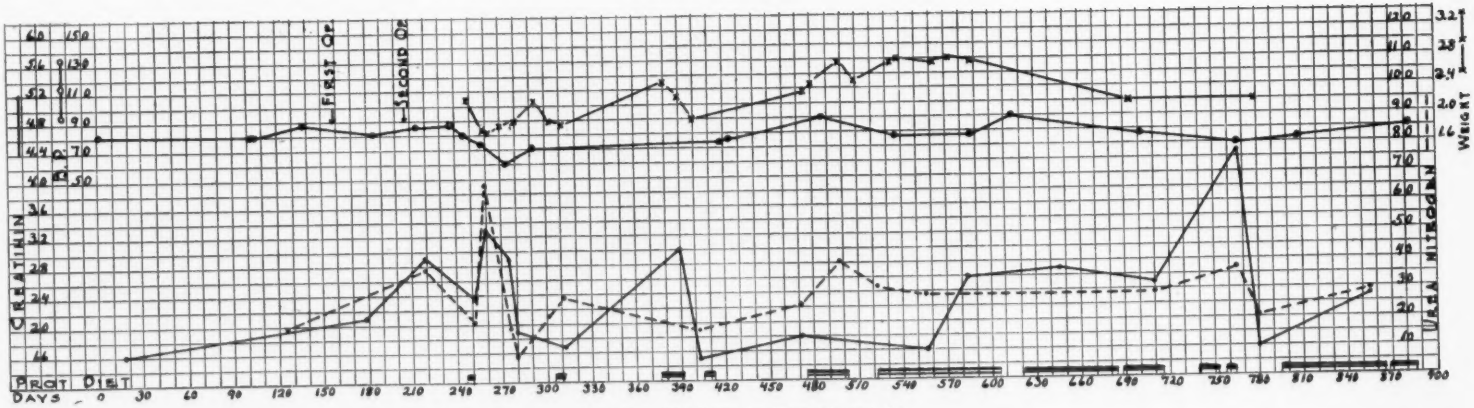

Fig. 12.—Findings in Experiment 44.

Day 540, albumin was + + +; specific gravity was 1.016; there were many pus cells in the sediment.

Day 600, the urine specimen showed albumin + and specific gravity 1.031; the sediment contained a few pus cells.

Day 607, the animal delivered young. She bled from the genital tract until Day 614. She became extremely ill during this time, but grew better rapidly as soon as the bleeding stopped. There was apparently some injury to the birth canal and rectum at the time of this delivery. The mucous membrane was observed to protrude at times and the parts always remained soiled thereafter.

Day 630, the right eye showed a purulent discharge originating in some lacerations of the eyelids.

Day 667, a twenty-four hour urine specimen had a volume of 265 cc.; there was a trace of albumin; specific gravity was 1.021; the sediment showed nothing abnormal.

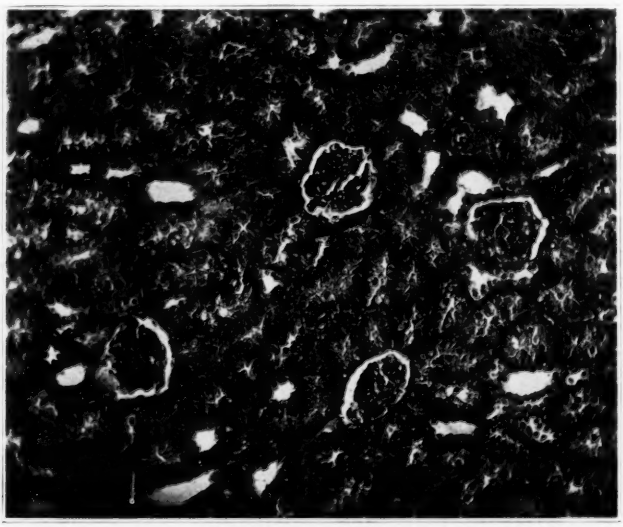

Fig. 13 (Experiment 44).—Section of wedge removed from left kidney at first operation; same magnification as Figure 14.

Day 669, a twenty-four hour specimen had a volume of 460 cc.; albumin was +; specific gravity was 1.023; the sediment contained nothing abnormal.

Day 779, the animal was pregnant.

Day 835, the fundi were examined for signs of arteriosclerosis and albuminuric retinitis; nothing abnormal was found.

Day 848, a twenty-four hour urine specimen had a volume of 151 cc.; albumin was +; specific gravity was 1.032; the sediment contained only triple phosphate crystals and epithelial cells.

Day 866, the fundi were examined with red-free and with white light; nothing abnormal was found.

Day 868, the animal delivered young, one of which lived for twenty-four hours. The remainder died promptly.

Day 887, she was killed by a blow on the head, 681 days after the removal of the right kidney. The total number of days on a high protein diet was 341.

At necropsy the right kidney was absent. The left was surrounded by a large quantity of fat and was bound down by adhesions; the weight was 12.25 Gm.; the cortex appeared wider than usual. The scar of operation was healed; the pelvis was normal. There were many atheromatous patches throughout the aorta, two dilatations with thinning of the wall being present in the thoracic portion. The entire vessel appeared widened. The heart, which weighed 9.1 Gm., was normal. The liver was normal. There was much omental, mesenteric and perirenal fat. The ovaries contained a number of small cysts.

Microscopically, the remnant of the left kidney showed marked hypertrophy of the glomeruli and tubules (Fig. 14). In the aorta there were marked atheromatous change in the intima and calcification in the media. The liver showed marked hydropic change, marked periportal infiltration of lymphocytes and some hyperplasia of the biliary ducts.

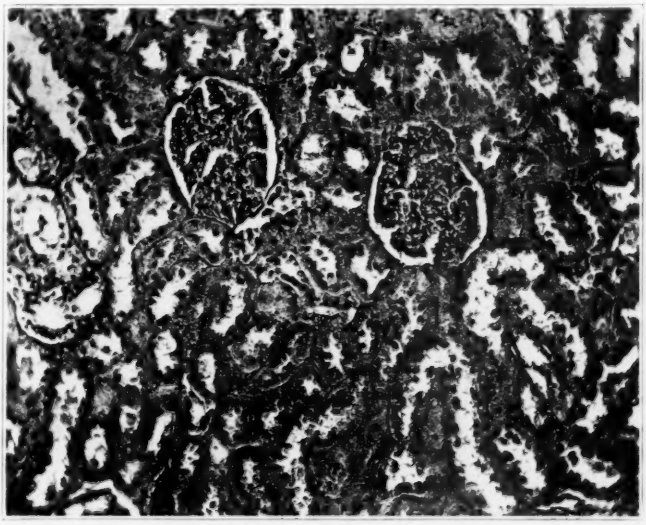

Fig. 14 (Experiment 44).—Remnant of left kidney 681 days after removal of right kidney and after 341 days of high protein feeding; hypertrophy of glomeruli and tubules (comparison should be made with Figure 6).

The abnormal findings were: hypertrophy of the left kidney; atherosclerosis with dilatation of the aorta; hydropic change in the liver, with biliary duct hyperplasia.

PROGRESS OF THE DIET GROUP

Rabbits 29 to 44 compose the diet group (Group 3). The group consumed a total of 522 pounds (236.8 Kg.) of roundsteak. The daily consumption averaged 87.2 Gm. per rabbit, or about 4 per cent of the body weight.

Rabbits 32 to 44 were all operated on. All recovered and in most cases were allowed to eat a normal diet until the concentration of the metabolites in the blood had returned to normal.

Rabbits 32 to 35 were operated on only once and deprived of 12 per cent or 50 per cent of kidney substance. These did not show an increase of creatinin above normal limits.

Rabbits 36 to 44 were subjected to removal of from 62 to 68 per cent of kidney substance. There was a moderate postoperative increase in blood metabolites. The creatinin values lay between 2.36 and 3.0 mg. per hundred cubic centimeters. The urea values rose to from 28 to 42 mg. per hundred cubic centimeters but were somewhat inconsistent. The return to normal values occurred in a time varying from twenty-six to 390 days.

The first rabbits given a high protein diet (Rabbits 36, 38 and 44) received the whites of hard boiled eggs after Newburgh and Clarkson.[3] They all refused to eat until driven by hunger. They fasted for several days before eating. All lost weight rapidly. All became constipated, passing only small amounts of feces once every two or three days. Rabbit 38 fasted for six days and at the end of that time was given a normal diet. It had eaten no egg whatever but showed some albumin in the urine and the sediment contained casts.[3] It lost 10 per cent of its body weight. Following this a normal rabbit was fasted two periods of five days each to determine the effect on the urine. Albumin was present on the fourth and fifth days in the first period and on the third day in the second period. These few observations prove nothing but indicate that the results of Newburgh and Clarkson may have been at least partially inanition phenomena or the results of constipation.

Rabbits 38 and 44 were later fed the meat bread constituting the high protein diet of this study. Whereas both lost weight on the egg diet both gained while eating the meat bread.

Weight.—In all experiments in which a number of weight determinations were made the animals exhibited a gain, except Rabbits 37 and 38 (protocols). These two lost slightly. In four others (Rabbits 32 to 35) the weights were disregarded as unreliable.

The ten animals which were still alive at the close of the experiments and which were killed had all been on the high protein diet until within twenty-four hours of death. All were fat. Each contained large deposits of fat in the omentum, mesentery and perirenal tissues. It cannot, therefore, be argued that any of the findings here recorded were due to inanition.

Blood Metabolites.—Many writers have described a rise in blood urea values following a high protein diet.[9] Such a rise occurred in the animals that were operated on. Subgroup D (65 per cent removed) ranged from normal to 74.8 mg. per hundred cubic centimeters of blood;

9. Chace and Rose, Folin, Denis and Seymour, and McLean (Footnote 5, first, second and third references).

Subgroup C (50 per cent removed) up to 43.4 mg.; Rabbit 33 in Subgroup B (12 per cent removed) showed 35 mg. per hundred cubic centimeters.

I have not seen described any similar rise in creatinin. In fact, the concentration of creatinin is said to be independent of the diet. In these experiments the creatinin also rose to high levels, which were maintained consistently. In fact, the creatinin was found to be a much more reliable index than the urea nitrogen, although somewhat less delicate.

The amount of kidney substance functioning seemed to determine definitely the concentration of the metabolites when the diet was given. Hewlett [10] and Chace and Rose [11] describe a rise in the concentration when much protein is given in the diet but do not make entirely clear the matter as to whether this rise occurs in normal individuals or only in those which are known to have a lowered renal function. McLean [6] states that the level is higher in nephritis than in normal persons but does not definitely say whether there is a rise in normal individuals.

The results of these experiments are definite on this point. The control animals not operated on showed no rise of either urea nitrogen or creatinin even after long periods of time on the diet (Rabbits 29, 30 and 31; Figs. 1 and 2). Only the animals having a reduced kidney filter showed a rise in concentration of metabolites. The rise was proportionate to the amount of kidney substance removed.

The rise appeared even in the case of Rabbit 32 from which only 12 per cent of the kidney substance had been removed. The urea nitrogen rose to 35 mg. after five days on the protein diet and twelve days after the operation. The blood of Rabbit 33 (the other rabbit in Subgroup B) unfortunately was not examined until nineteen days after operation. If there had been a rise in the first two weeks, the values had returned to normal when the blood was examined. It is possible that the hypertrophy which is known to occur may overcome entirely deficiencies as small as 12 per cent.

The two animals in Subgroup C (50 per cent of kidney removed) both showed definite rises in the values of both creatinin and urea. These values were intermediate between those obtained in Subgroups B and D. The creatinin in the case of Rabbit 34 rose to 1.8 mg., in Rabbit 35 to 2.5 mg. The urea nitrogen in Rabbit 34 rose to 35.2 mg. and in Rabbit 35 to 43.4 mg. (Table 1).

Subgroup D (Rabbits 37 to 44; 65 per cent plus removed) gave the highest values, having lost most kidney substance (protocols and charts). The creatinins varied from 2.5 to 4.3 mg. and the urea nitro-

10. Hewlett, Gilbert and Wickett (Footnote 5, fourth reference).
11. Chace and Rose (Footnote 5, first reference).

gen from normal to 74.8 mg. while on the protein diet; yet the animals all appeared healthy and were active at all times. Rabbits 39 and 40 eventually developed calculi and hydronephrosis and exhibited much higher values, but remained in good health until these complications intervened.

All groups returned to normal or to much reduced concentrations each time that a normal diet was substituted. It usually required only a few days for this change to take place, and again only a few days for a return to high levels when the protein diet was given.

These findings indicate that the removal of the kidney substance raises the threshold values of creatinin and urea. The rise in the threshold varies directly with the amount removed.

Blood Pressure.—The blood pressure in the diet group did not vary from that of the controls that were operated on. In general, the blood pressure of all the animals remained within normal limits at all times whether they were on a normal or a protein diet, irrespective of the amount of kidney tissue functioning (protocols and charts of Rabbits 29 to 44). Even in the case of Rabbit 44, which survived an attack of pyelonephritis, there was no hypertension (protocol of Day 258).

It seems certain that neither a reduction of the kidney nor a high concentration of urea and creatinin in the blood raises the blood pressure. It is inferred that when hypertension occurs in chronic glomerulonephritis, there is some other factor acting to cause the increased blood pressure.

Fundi.—The fundi were examined from time to time as in the controls operated on. In addition to the usual examination with the Welch-Allyn ophthalmoscope, the red-free light [12] was used in ten cases (Rabbits 30 to 35 and 42 to 44). None of the common signs of arteriosclerosis or albuminuric retinitis were observed at any time by either method.

Urine.—There were no striking urinary changes following the high protein feeding. Seven of the sixteen diet animals showed albumin on different occasions. These seven were Rabbits 30 (normal control) and 39 to 44. The amounts were small except in the case of Rabbit 44, which at one time developed a pyelonephritis with pus and blood in the urine. Usually albumin was absent.

Pregnancy.—The females all became pregnant frequently. In general, they carried their litters to term, but the young did not live after delivery. During the time when the diet experiments were carried out (over a year) only three young survived. These were delivered by

12. Vogt, Alfred: Ueber eine vertikale Streifung, welche an der Vorderfläche der Netzhaut jüngerer Individuen im Rotfreien Licht wahrgenommen wird, Klin. Monatsbl. f. Augenh. **60**:47, 1919.

Rabbit 43. Every effort was made to keep the young alive but, with the exception just mentioned, all died in from twenty-four to forty-eight hours.

The reason for the failure of the young to survive is not clear. It may be that the diet was lacking in some essential factor although it was thought to be complete when first prepared.

The fresh lettuce given at weekly intervals contained vitamins A, B and C but may have been insufficient in quantity to supply the young with necessary accessory food factors. The lean beef used contained all three of these vitamins but at least the antiscorbutic factor was destroyed by the baking. This factor was probably absent entirely from the meat bread and supplied only by the lettuce.[13]

NECROPSY FINDINGS

Kidney.—Rabbits 39 and 40 died from obstruction by renal calculus. These showed hydronephrosis grossly, and microscopically gave the typical picture of obstruction. No microscopic examination was obtained in the case of Rabbit 29.

The kidneys of the remaining thirteen in the diet group were carefully examined both grossly and microscopically. The only significant change found was hypertrophy.

Hypertrophy occurred in all. Even the kidneys taken from the controls that were not operated on (Rabbits 30 and 31) were hypertrophied.

The average weight of twenty-six normal kidneys removed in toto at operation was 9 Gm. Only three weighed as much as 11 and one only as much as 13 Gm. The average weight of the two kidneys removed from Rabbit 30 at necropsy was 11.5 Gm. The weight of the right and the left kidneys from Rabbit 31 averaged 14.2 Gm. (Table 4).

The left kidney was removed in part or completely from Subgroups B and C. At necropsy the weights of the right kidneys averaged 12.5 Gm., which represents a distinct hypertrophy.

In Subgroup D the kidney remnants of six of the nine weighed as much or more than the other kidney previously removed in toto. In the cases of Rabbits 43 and 44 the hypertrophy was great, in both cases estimated at more than 100 per cent.

Hypertrophy also occurred in the controls that were operated on. For that reason it is clear that a degree of hypertrophy follows the operative reduction. That the diet has an added effect also seems reasonably certain. The hypertrophy in general was greater in the diet animals

13. Owing to the erection of a new hospital building during this time the animals were kept in temporary pens unsuited to the raising of young. This may have been a factor in the death of the young.

than in the operated controls and moreover the controls not operated on which ate the protein diet developed hypertrophy.

Microscopically, there was no evidence of tubular or glomerular injury resulting from the diet. The operative specimens were examined in all cases and compared with the portions removed at necropsy, in some cases more than two years later. The same kidney (left) was available for microscopic study both before and after the diet experiments in most cases. Unless complications had intervened, there were no changes except hypertrophy and, in some cases, an increase in the extent of spontaneous nephritis.

Figure 13 is a photograph of the wedge removed from the left kidney of Rabbit 44 on Day 158. Figure 14 shows a section from the remnant of the left kidney found at necropsy 729 days (two years)

TABLE 4.—*Summary of Operative Procedures and Necropsies in Diet Animals*

Rabbit	Days Survived After Operations	Days on High Protein Diet	Weight of of Piece of Left Kidney Excised	Weight of Right Kidney Excised	Percentage of Kidney Substance Remaining	Weight of Remnant of Left Kidney	Microscopic Findings in Remnant
29	...	48	....	....	100	....	
30	...	357	....	11.4	100	11.6	Normal
31	...	324	....	14.5	100	13.9	Normal
32	63	52	1.85	10.7	88	5.9	Normal
33	90	52	1.9	12.4	88	8.4	Normal
34	97	88	....	14.7	50	9.1	Normal
35	113	88	....	12.3	50	....	Pigment in medulla
36	64	5	Infarct	11.9	35*	7.8	Normal
37	160	23	Infarct	8.2	35*	....	
38	449	17	2.0	7.3	36	....	
39	301	128	Infarct	11.4	35*	11.5	Hypertrophy of tubules and dilatation
40	348	238	2.3	8.3	36	14.5	Pyelonephritis
41	469	325	2.55	8.05	34	10.2	Hypertrophy of tubules and glomeruli
42	488	325	Infarct	7.1	35*	9.1	Normal
43	490	325	Infarct	8.3	35*	15.7	Hypertrophy of tubules and glomeruli
44	681	341	2.35	8.35	35.9	12.25	Hypertrophy of tubules and glomeruli

* Approximate only.

after operation. The remnant had carried on the work of both kidneys for 681 days and had been subjected to the effects of protein feeding for 341 days. There was a marked hypertrophy of the glomeruli and tubules but no other change.

The increased number of scars from spontaneous nephritis found at necropsy is explained on the basis of the increase in age of the animals.

Aorta.—In the vascular system the only pathologic condition found was confined to the aorta and the aortic valves. The lesion found was a marked atherosclerosis.

This sclerosis did not extend to the small arteries or arterioles. The small arteries and arterioles of the lungs, heart, liver, spleen, small intestines, kidneys, tubes, ovaries and voluntary muscle were carefully examined in each case for thickening and degeneration. None was

found. This fact may explain the lack of fundus changes and the failure of the blood pressure to rise. Hypertension in case of human nephritis appears in those cases which develop sclerosis in the smaller vessels. The fundus changes in nephritis, according to Umber,[14] are due to vascular disease and these changes would not occur in the presence of normal vessels.

The aorta was carefully described in thirteen of the sixteen in the diet group (protocols). Four contained extensive and marked lesions. Four showed a moderate atheromatous change with occasional small spots of calcification. Four were normal; one other was nearly normal.

In the case of Rabbit 39, the aorta was dilated through the greater portion of its length and was so calcified that it fractured repeatedly during the manipulations necessary to remove it. In Rabbits 31 and 44 localized dilatations developed. These were in fact broad, shallow, saccular aneurysms. In both cases these dilatations occurred in calcified areas located in the arch or upper thoracic aorta.

The sclerotic changes in the aorta did not seem to bear any relation to the amount of kidney substance functioning. Two of the normal aortas were found in Subgroup D (65 per cent removed), one in Subgroup C (50 per cent removed) and one in Subgroup B (12 per cent removed). Both of the controls not operated on presented sclerosis. In one of them (Rabbit 31, Fig. 3) the disease was far advanced; just as far advanced as in Rabbit 43, which had undergone both operations (Fig. 11).

The length of time on the protein diet seemed to bear a direct relation. In general, the extensive changes occurred in those which had eaten meat bread over a long period of time. This did not hold true in each case. Rabbits 41 and 42 were both on the protein diet for 325 days. Neither developed much injury. The aorta in the former was only moderately pathologic and in the latter was entirely normal. Such variations as these are best explained on the ground of individual susceptibility.

The pathologic changes were typical, microscopically, of arteriosclerosis. The atheromatous areas proved to be composed of swelling, fatty deposits and degenerative changes in the intima. The calcium deposits lay in the media. In many areas, the media contained degenerated fibers with, as yet, no deposit of calcium (Figs. 11 and 3). Newburgh and Clarkson[3] have described similar changes after protein feeding.

The controls operated on developed no aortic lesions, even those which died from renal insufficiency. This fact indicates that the marked arteriosclerosis found in the diet animals was due to the diet. It can-

14. Umber, F.: Heutige Standpunkt in der Pathologic und Therapic der Nierenkrankheiten, Deutsch. med. Wchnschr. **49**:369 (March 23) 1923.

not be argued with certainty that the protein caused the damage because there are other possible factors which have not been controlled. It is even possible that we are dealing with a deficiency disease.

Liver.—The liver was found to be pathologic in all of the diet animals except in the case of Rabbit 36, which had been on the diet only five days.

The changes in all cases were microscopic and in most were represented by a hydropic condition of the liver cells. This was in contrast to the fatty metamorphosis found in the liver cords in a number of the controls operated on. The hydropic change was most marked at the centers of the lobules, but in many included the entire lobule (Rabbits 30 and 32) and was extensive in the extreme. Its exact nature and cause is not clear. It occurred in both the animals operated on and those not operated on and is considered a result of the diet.

Rabbit 35 presented fatty change and Rabbit 39 a number of areas of necrosis. The necrosis was at the centers of the lobules. In Rabbit 40 there was a periportal lymphocytic infiltration. This could be explained on the basis of the pyelonephritis which accompanied the hydronephrosis in this case.

SUMMARY

The purpose of this study was (*a*) to determine the blood pressure changes in low renal function or insufficiency and (*b*) to determine the possible influence of high or low protein diet on the course of chronic renal disease.

Thirty-eight rabbits were operated on before any were placed on a high protein diet. Of these twenty-nine died without having been on a protein diet. All except four showed microscopic or gross signs of obstruction at necropsy.

The four that died of renal insufficiency without signs of obstruction all showed diffuse hydropic change in the kidney remnant. This change was most extreme in two cases. It is considered that this hydropic degeneration represents the results in the kidney of renal insufficiency without other complications. It is probably due to overactivity.

Following the operative procedures in which over 60 per cent of kidney substance was destroyed, there was a retention of creatinin and urea nitrogen in the blood. This became progressively greater until death in those which died, but decreased in those which recovered until the values again became normal.

There was no increase in the blood pressure following the reduction of kidney substance, even in those which died from a slowly progressive renal insufficiency.

A high protein diet containing over 30 per cent of protein was fed to sixteen rabbits over a long period of time, in a number of cases about a year. Three of these rabbits had not been operated on; two had

lost 12 per cent of kidney substance; two had lost 50 per cent, and the remaining nine had lost from 60 to 70 per cent of kidney substance.

The only change observed in the kidneys which might be attributed to the diet was a hypertrophy. There were no degenerative or inflammatory changes found except those of spontaneous nephritis.

All the diet animals showed approximately normal concentrations of blood metabolites when the high protein feeding was begun. The normal controls developed no increase while on the diet. All the animals operated on developed a retention of creatinin and urea nitrogen the degree of which was proportionate to the quantity of kidney substance removed.

The high concentration of the metabolites did not seem to injure the health of the rabbits. They seemed to thrive on the diet. The young, however, did not survive.

There was no increase in the blood pressure during the periods when high protein diet was given.

The liver in all the diet animals showed an extensive hydropic change. The cause of this change is not clear.

The aorta in many showed signs of extensive atherosclerosis, with thinning of the walls, dilatation and calcium deposits.

CONCLUSIONS

1. Removal of two thirds of the kidney substance in rabbits results in a retention in the blood of urea and creatinin, which either increases progressively in intensity until death results, or decreases progressively until normal values are once more attained.

2. If the renal insufficiency is not progressive, the kidney remnant undergoes hypertrophy.

3. A high protein diet causes hypertrophy of the kidneys in normal rabbits not operated on.

4. A high protein diet causes no further change in the kidney remnant than to further augment the hypertrophy.

5. A high protein diet causes a retention of creatinin and urea in the blood in rabbits from which a portion of kidney substance has been removed. It is proportionate to the amount of kidney which has been removed. The retention does not occur in normal rabbits.

6. Hypertension is not caused in rabbits by renal insufficiency per se; nor by a high protein diet even in the presence of a low renal function; nor by prolonged retention of creatinin and urea in the blood.

7. The high protein diet here given results in a marked atherosclerosis of the aorta which does not extend to the small arteries. These changes in the aorta do not result from low renal function or from prolonged retention of creatinin and urea in the blood.

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HEMOCHROMATOSIS AND CHRONIC POISONING WITH COPPER*

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Hemochromatosis is not so rare a disease as is generally supposed but the clinician has difficulty in recognizing it until the final stage when the three characteristic signs, cirrhosis of the liver, diabetes mellitus and pigmentation of the skin, are all evident. As for the earlier stages even the pathologist may occasionally overlook them.

This paper will consist of a general introduction to the subject of cirrhosis, of which pigment cirrhosis forms one type, and then of a discussion of different aspects of hemochromatosis under three headings, which are arranged in logical order although this is not the way in which the work was originally done. I had been trying for many years to discover the cause of so-called alcoholic cirrhosis. While testing out the injurious action of copper, among many other sub-

Incidence and Classification

	Number	Per Cent
Postmortem examinations (more than 1,000 in children)	5,400	....
Well marked cases of cirrhosis	250	4.63
Classification of Cirrhoses:		
1. Alcoholic cirrhosis	114	45.60
2. Pigment cirrhosis	19	7.60
Types 1 and 2 combined (the lesions of alcoholic and pigment cirrhosis both present)	50	20.00
3. Syphilitic cirrhosis	15	6.00
4. Infectious cirrhosis	13	5.20
5. Toxic cirrhosis	10	4.00
Old healed cirrhosis, type not determinable	29	11.60

stances, I found that with it I could produce pigmentation and cirrhosis of the liver in rabbits in from three to twelve months, according to the size of the dose. This observation led to a more thorough histologic study of the lesions of hemochromatosis and later to a search for possible sources of copper poisoning to which man might be exposed.

TYPES OF CIRRHOSIS

An analysis of all the cases of cirrhosis of the liver which have come to postmortem examination at the Boston City Hospital during the last twenty-eight years shows that they can be divided into five different types, each with its own characteristic primary cell lesion and resulting gross appearance. The accompanying table shows the number of cases available for study and the frequency of occurrence of each type.

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*Tenth Mellon Lecture before the Society for Biological Research of the University of Pittsburgh School of Medicine, April 30, 1925. Repeated as Hanna Lecture at the one hundred and eighty-third regular meeting of the Clinical and Pathological Section, Academy of Medicine of Cleveland, May 1, 1925.

A brief survey of the various kinds of lesions terminating in cirrhosis will make the subject of hemochromatosis easier to understand and more interesting. The different types will be presented not in the order of their frequency but from the pathogenic point of view. The first two are of infectious origin. By this term I mean that the microorganisms causing them are present in the lesions. The other three are due to injurious chemical agents brought to the liver by the blood.

Infectious Cirrhosis; Biliary Cirrhosis.—The first type is due to ascending infection of the bile ducts by the colon bacillus (perhaps also sometimes by related organisms) which gives rise to a cholangeitis.

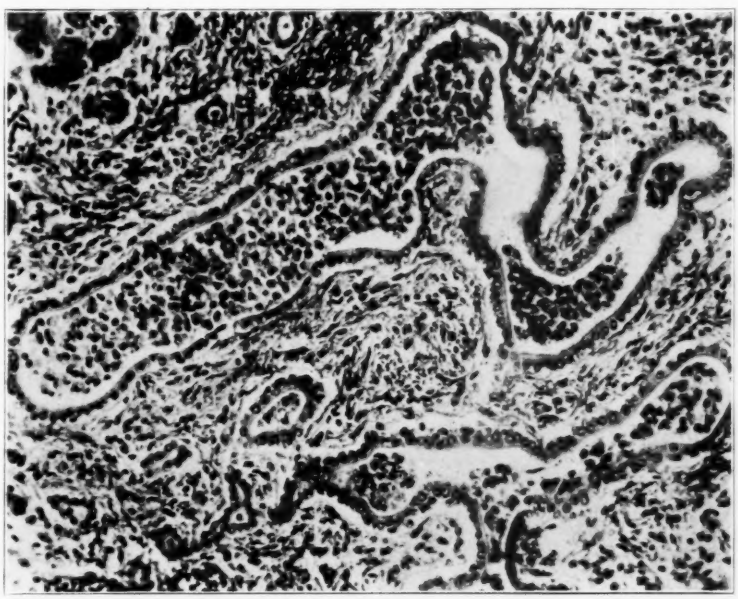

Fig. 1.—Acute cholangeitis.

The bacilli spread quickly throughout the liver causing acute inflammation within and around the bile ducts with destruction of the adjoining liver cells and fibroblasts of the stroma, followed by active regeneration of the connective tissue cells. In the early stages of the process the liver may be doubled in size. Obstruction of the bile ducts by exudate leads quickly to jaundice. The organisms may die out and repair with contraction of the liver follow. Sometimes the process recurs.

In the early stages the surface of the liver is smooth; later it becomes finely granular. The most distinctive and characteristic feature of the later stage of the lesion is the broad zone of connective tissue

around the portal vessels, the liver cells around the hepatic veins in the centers of the lobules at the same time being intact. The original lobulation is not lost as it is for example in alcoholic cirrhosis.

This type of cirrhosis is comparatively rare so that a series of cases for special study, especially from the pathogenic point of view, is difficult to obtain. It seems probable, judging from the clinical descriptions of his case, that this is the variety of cirrhosis described by Hanot and known under his name. From the pathologic descriptions of his livers nothing can be made out and so far I have been unable to obtain from any pathologist in France a section of what he regards as Hanot's cirrhosis.

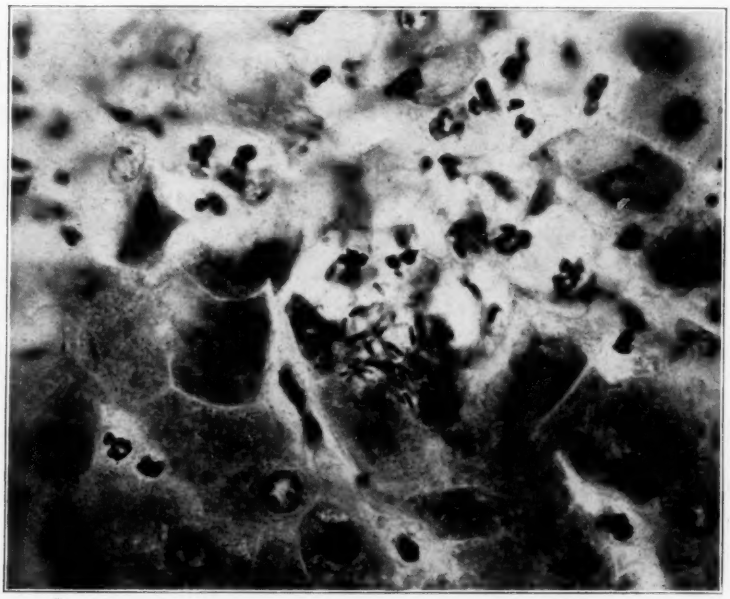

Fig. 2.—Colon bacillus invading liver lobule; necrosis and acute inflammatory reaction.

Syphilitic Cirrhosis.—This type is due to the presence of *Spirochaeta pallida* in the lesions. The injurious action of the organism is exerted chiefly or entirely on the fibroblasts of the stroma. Necrosis and regeneration of the connective tissue cells occur, followed by contraction of the collagen fibrils and compression, atrophy and disappearance of the included liver cells. The result is a more or less diffuse sclerosis. The lesion is complicated, occasionally in congenital syphilis, quite regularly in the acquired disease, by proliferation of the intimal tissue in arteries leading to obliteration of the lumen, followed by

necrosis (infarct, commonly called gumma) of the tissue no longer supplied with blood. Organization of multiple necrotic foci by granulation tissue followed by contraction causes deep scars which sometimes divide the liver into many lobes. The condition is characteristic and is recognized in the appropriately descriptive term hepar lobatum, in my experience, at least, a comparatively rare condition.

I was called over to the surgical amphitheater one day before Dr. Lahey's fourth year class. He was operating on a woman for ulcer of the duodenum, a diagnosis made by certain students who had examined the patient and concurred in by him. Nothing was found at operation except an unusual condition of the liver which he had never

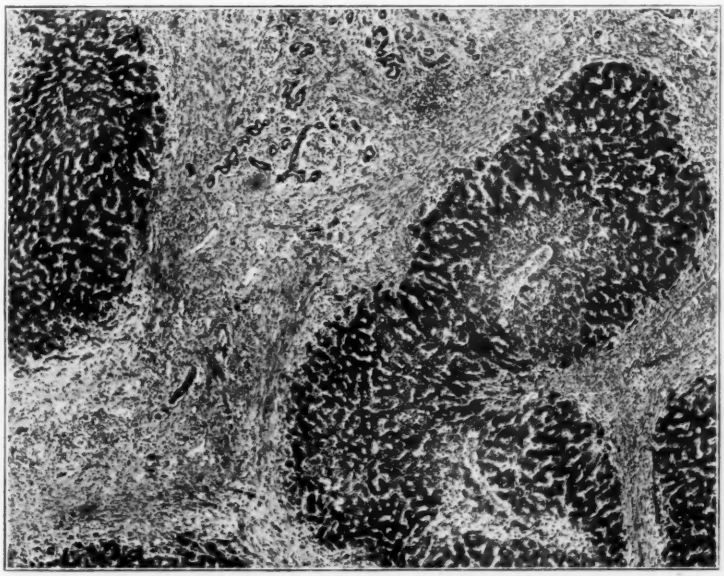

Fig. 3.—Biliary cirrhosis; lesion located around bile ducts; liver cells around hepatic veins intact.

seen and did not recognize. After examining the liver tissue through the incision I asked what the Wassermann test had been. "Negative," the class answered. I took another look and said that the appearance was typical of syphilis of the liver and could be due to nothing else. I asked Dr. Lahey to cut me out a piece, not from an elevated part but from a scar. He answered that he didn't like to but that he would. On one face of the wedge shaped piece excised I was able to show to the class at once a small, healing gumma and on the other side two. On my return to the laboratory I sent over to the clinic

Fig. 4.—Biliary cirrhosis; gross appearance externally and on section.

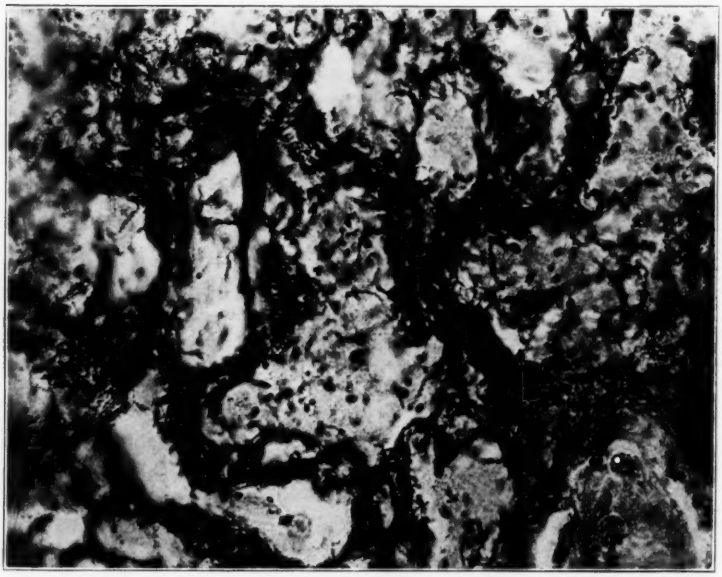

Fig. 5.—Masses of *Spirochaeta pallida* in connective tissue stroma of liver causing sclerosis in a new-born child.

a photograph of a typical case of hepar lobatum in order to impress the lesion more deeply on the students.

The three other types of cirrhosis are of toxic origin.

Toxic Cirrhosis; Cirrhosis of Acute Toxic Origin.—A variety of injurious agents can cause this type of cirrhosis, for example, phosphorus, arsenic (frequently following treatment with arsphenamin), chloroform, carbon tetrachlorid and the toxin derived from certain streptococci. The destructive lesion, extensive necrosis of the liver cells, appears to be always of acute origin. The necrosis affects first the cells in the centers of the lobules and spreads peripherally. If the lesion is not too extensive (central necrosis), removal of the necrotic

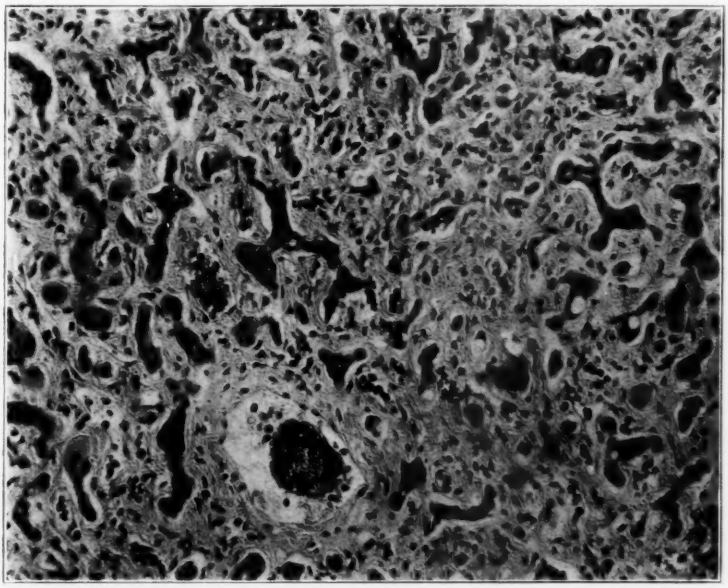

Fig. 6.—Diffuse sclerosis of liver due to acquired syphilis in an adult.

cells and regeneration of them may occur within ten days. If all the cells in a lobule are killed, no regeneration of liver cells takes place. The bile duct epithelium proliferates to a slight extent and then stops: it cannot form liver cells. They can regenerate only from liver cells.

In cirrhosis of acute toxic origin all the liver cells in many lobules may be destroyed. After the necrotic cells have been removed through the digestive action of leukocytes, these foci of stroma, sinusoids and bile ducts shrink (acute red atrophy). In other foci where liver cells remain rapid regeneration takes place and the new formed liver cells are stained yellow by bile.

Next to the syphilitic liver the liver of acute toxic cirrhosis may present the greatest irregularity of surface, projecting nodules of all sizes due to regeneration of liver cells and depressed areas due to condensation of stroma where the cells have disappeared, but there is never the deep scarring which results from organized gummas. At other times the liver may closely resemble that of alcoholic cirrhosis but the nodules are usually more irregular in size.

Excessive regeneration of liver cells sometimes results in the formation of nodules one or more centimeters in diameter which have been called adenomas. In these nodules no lobular arrangement is evident and bile ducts are absent.

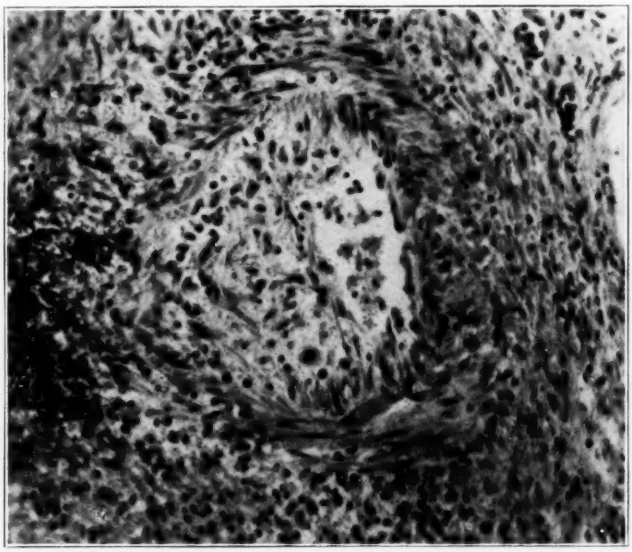

Fig. 7.—Syphilitic endarteritis (from same liver as shown in Figures 6 and 8) leading to occlusion of lumen and formation of infarct (gumma); one mitotic figure present.

In the atrophied portions where there are no liver cells left the original lobular arrangement, much shrunken, can almost always be made out even if these parts have been stretched and pulled out of shape by excessive regeneration of liver cells in other parts. It is the only type of cirrhosis in which the condition occurs.

The early stage of this type of cirrhosis is often recognized clinically owing to the rapid decrease in the size of the liver, often to a half or even a third of the original volume. It is usually called acute yellow atrophy of the liver owing to its greatly diminished size and

to the yellow color of the regenerated parts: acute red atrophy is the preferable term.

The lesions in the two remaining types of cirrhosis are chronic in type and take years in which to produce their effects.

Alcoholic Cirrhosis.—The cell lesion is characteristic and consists of an acidophilic reticulum which forms in the liver cells around the nuclei, apparently from coalescence of granules. It stains intensely with eosin by the eosin-methylene blue method after fixation in Zenker's fluid. In time (months to years) the cells die and are removed by the digestive action of polymorphonuclear and endothelial leukocytes.

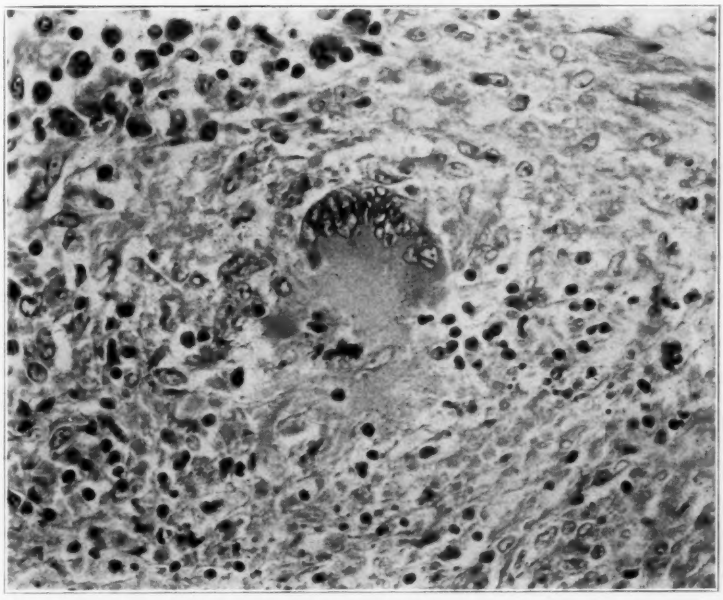

Fig. 8.—Foreign body giant cell at edge of gumma (same liver as shown in Figures 6 and 7).

Regeneration from other liver cells takes place diffusely and in foci. Where the cells die condensation of the stroma (sclerosis) occurs; where regeneration of liver cells takes place new stroma is formed, as in a tumor. In this way the amount of connective tissue in the liver is gradually increased as one crop of cells after another is killed off.

The gross picture of alcoholic cirrhosis is fairly characteristic. The external surface is finely to coarsely granular. The elevated portions represent the regenerated islands and groups of islands of liver cells, the depressed portions the contracted stroma. In the early stages

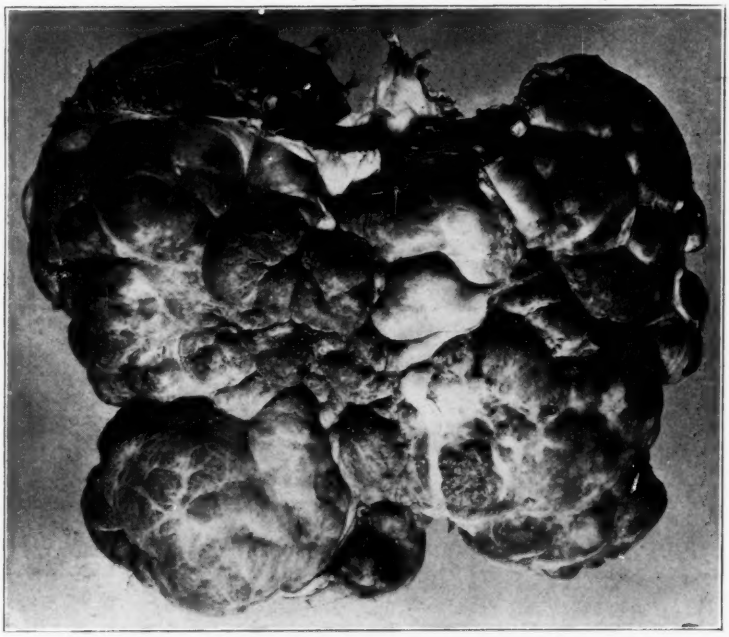

Fig. 9.—Typical syphilitic cirrhosis (hepar lobatum).

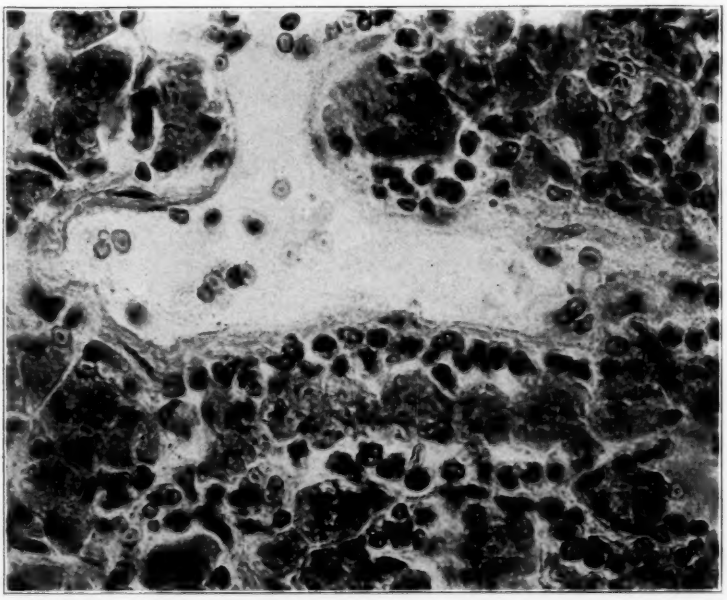

Fig. 10.—Necrosis of liver cells around hepatic vein; invasion by polymorphonuclear and endothelial leukocytes.

of the process the lesion is often complicated by marked fatty infiltration of the liver cells leading to great increase in the size of the liver.

We came now to the fifth type of cirrhosis which forms the main subject of this paper. It is probably the slowest of the five types in developing, requiring apparently at least fifteen years.

Pigment Cirrhosis, Hemochromatosis, Bronzed Diabetes.—I shall speak of the macroscopic appearance of the liver first and then take up in detail the development of the lesion that leads to the gross picture. In many ways the liver, except for the rusty to dark brown color, resembles that seen in alcoholic cirrhosis. It may be larger or smaller

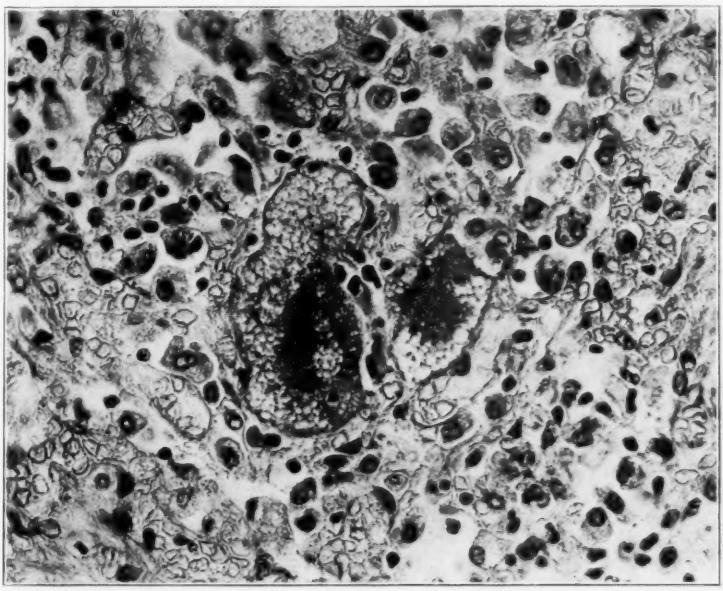

Fig. 11.—Acute yellow atrophy; most of cells killed and removed by digestive action of leukocytes; a few liver cells left from which regeneration may take place.

than normal according to the duration of the process [1] and its surface may be smooth to finely and coarsely granular depending also on the stage of the disease. It differs in one respect, however, from the alcoholic type: the lesion is rarely complicated by fatty infiltration, at least to any extent, and therefore the weight of the liver is never so great as it may be with the latter.

1. Mills, E. S.: Hemochromatosis with Special Reference to Its Frequency and to Its Occurrence in Women, Arch. Int. Med. **34**:292 (Sept.) 1924.

Before proceeding to the histology of hemochromatosis I wish to mention certain effects that all five types of cirrhosis have in common. Contraction of the connective tissue in the foci of sclerosis leads to constriction of the blood vessels and of the bile ducts. Obstruction of the portal circulation causes distention and enlargement of the spleen, congestion of the gastro-intestinal tract and ascites. Dilatation of veins to effect a collateral circulation frequently leads to the production of varices in the esophageal plexus. Rupture of one of these dilated veins may result in sudden death from extensive hemorrhage into the stomach. Obstruction to the bile ducts leads to focal bile stasis within the liver and also to jaundice.

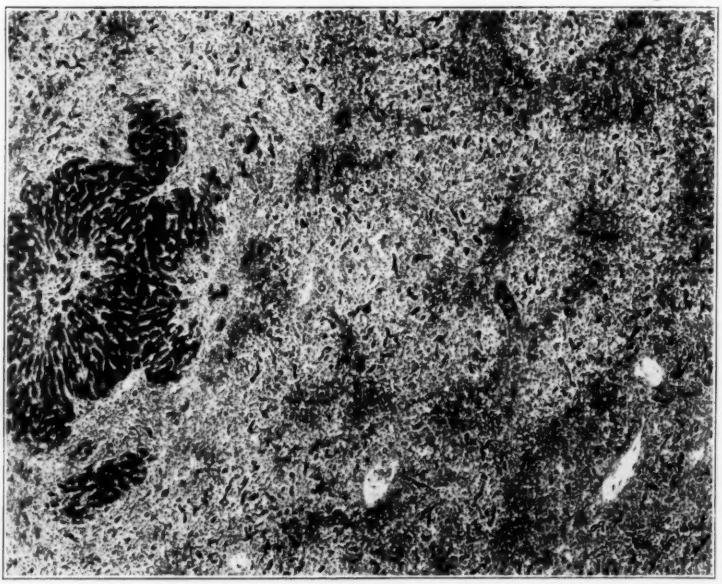

Fig. 12.—Cirrhosis following acute yellow atrophy; in the lobules where all the liver cells were killed no new formation of them takes place.

THE HISTOLOGIC LESION OF HEMOCHROMATOSIS

From the pathologic point of view hemochromatosis began with von Recklinghausen. He gave the disease its name and observed that two yellow pigments were present in the lesions, hemosiderin, which gives the reactions for iron, and another which does not and which he called hemofuscin. He did not recognize or suggest a relationship between these two pigments although he believed them both to be derived from hemoglobin.

As the result of a careful study of a large series of cases of pigment cirrhosis and with better technical methods of fixing and staining at our command, Parker, Nye and I [2] were able to demonstrate that hemofuscin is the first pigment to be deposited in the liver and other cells and that in the course of time it is slowly changed to hemosiderin. The method of demonstrating the relationship is simple.

In pigment cirrhosis, as in the alcoholic type, there is a constant destruction of liver cells and an equally constant regeneration of them provided the process is at all active. The newly formed cells may be scattered diffusely among the older pigmented cells but usually occur

Fig. 13.—Regeneration following central necrosis complicated by chronic passive congestion.

in islands up to a millimeter or more in diameter. At first the new cells contain no pigment; then gradually granules of hemofuscin appear. They stain readily and intensely with basic aniline dyes, especially with a dilute solution of fuchsin in 50 per cent alcohol. In the course of time (months to years) these granules lose this staining property and give the reactions for iron.

2. Mallory, F. B.; Parker, Frederic, Jr.: and Nye, R. N.: J. M. Res. **42**:461, 1921.

Hemofuscin deposited in fibroblasts and smooth muscle cells is usually changed with great slowness or not at all to hemosiderin. Consequently, this pigment can be found in these cells when, owing to the inactivity of the process, none is present in the parenchymatous cells of the liver or other organs because it has been changed to hemosiderin and gradually dissolved and eliminated.

Fig. 14.—Cirrhosis of acute toxic origin containing islands of excessive regeneration, so-called adenomas.

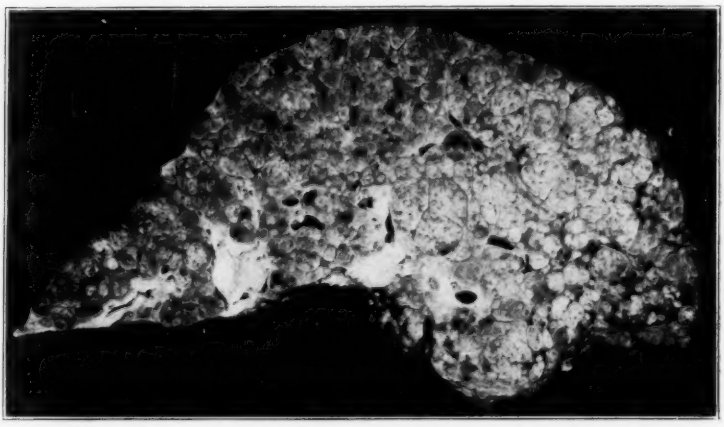

Fig. 15.—Cirrhosis of acute toxic origin in a girl, aged 15 years.

The primary essential lesion of pigment cirrhosis may be stated, therefore, to be the deposit of a yellow pigment, hemofuscin, in the parenchymatous and other cells of the liver. Like the acidophilic reticulum of alcoholic cirrhosis this deposit of hemofuscin is the "finger print of crime" left by an injurious agent, the identity of which we wish to ascertain. In time hemofuscin changes to hemosiderin. The

accumulation of either pigment beyond a limited degree causes necrosis of certain parenchymatous cells. This starts regeneration and the process continues until the patient dies from the effects of the process itself or from secondary complications.

The cell changes in the pancreas are similar to those in the liver: deposit of pigment, necrosis of acinous and islet cells, regeneration. The physiologic result is the production eventually of diabetes mellitus. It is on account of this association of diabetes with pigmentation of the skin that the French have applied the term bronzed diabetes to hemochromatosis.

In the suprarenals the pigment is deposited chiefly in the outer layer of the cortex. It has been claimed that the normal pigments of the

Fig. 16.—Cirrhosis of acute toxic origin in a boy, aged 13 years.

body, and especially that in the smooth muscle cells of the gastrointestinal tract, are increased in hemochromatosis. This would suggest that the lesion in the suprarenals might play some part in this increase were it not that proper staining of these smooth muscle cells shows the pigment to be hemofuscin except so far as it has been changed to hemosiderin.

The pigmentation of the skin is of great aid in rendering possible a positive diagnosis of hemochromatosis during life because the iron reactions can be made on sections of an excised piece.

The lesions in the kidneys are never marked, consisting chiefly of pigmentation of renal cells in the cortex. As the cells are killed off they desquamate, are washed out by the urine and are replaced by

regeneration. There is no accumulation of pigment in endothelial leukocytes in the organ and no sclerosis.

Pigment is deposited in cells all over the body, in the upper abdominal lymph nodes, in the heart, thyroid, brain, hypophysis, etc., but the effects are not serious because the patient dies as a result of the lesions in the liver and pancreas before they can develop in these other organs to any great degree.

The essential lesion of hemochromatosis may be summed up, therefore, as the deposit of hemofuscin in parenchymatous and other cells of the liver, pancreas, suprarenals and other organs and tissues, the change

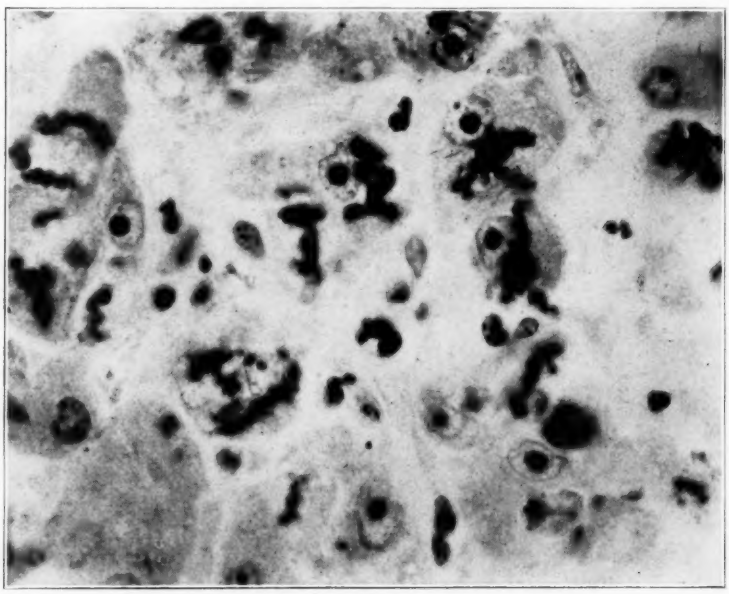

Fig. 17.—Liver cells showing hyaline reticulum characteristic of alcoholic cirrhosis.

of hemofuscin to hemosiderin through the metabolic activity of these cells, necrosis and regeneration of some of the parenchymatous cells, especially in the liver and in the pancreas, condensation of the stroma when all the parenchymatous cells in certain areas have been destroyed, and the formation of new stroma in the islands of regeneration just as in a tumor. The final result is sclerosis of the liver and pancreas with the consequent physiologic effects, ascites, jaundice and diabetes.

CHRONIC POISONING WITH COPPER

Several years ago I found out that acetate of copper added to the food of rabbits produced pigment cirrhosis in from three to twelve

months according to the size of the dose. Eventually jaundice would develop and then the animals died quickly. One rabbit that was given small doses lived almost four years. The liver was finely granular, and deep olive brown owing to pigmentation and jaundice, but showed a number of small white granules of regenerated nonpigmented cells.

The pigment in the early lesions consists entirely of hemofuscin granules which stain intensely with basic fuchsin. Necrotic cells and mitotic figures are fairly numerous. The lesion is always most marked at the peripheries of the lobules and as the cells are killed off the pigment is taken up by endothelial leukocytes which tend to collect in the connective tissue around the portal vessels. Pigment also occurs in

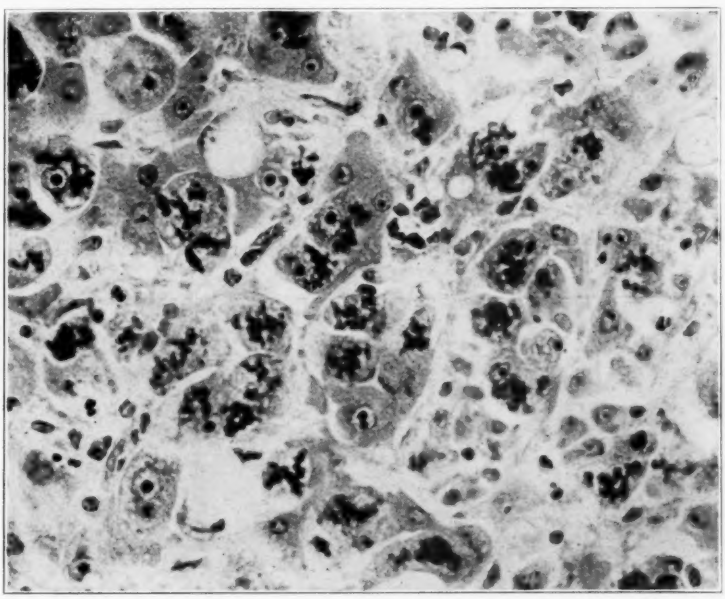

Fig. 18.—Many liver cells containing hyaline reticulum: some have undergone necrosis and are being dissolved by endothelial leukocytes.

some of the renal cells and in the bone marrow, and in the animals that live longest is found in slight amount in the heart and in the pancreas.

Similar lesions can be produced in the monkey. In one small variety from South America the hemofuscin granules were changing to hemosiderin at the end of five and one-half months. In the rabbit the change is moderate even at the end of nearly four years.

The sheep was found very susceptible to copper poisoning, requiring no larger doses than the rabbit. The liver at the end of one year showed beginning cirrhosis, considerable hemofuscin and a slight amount of

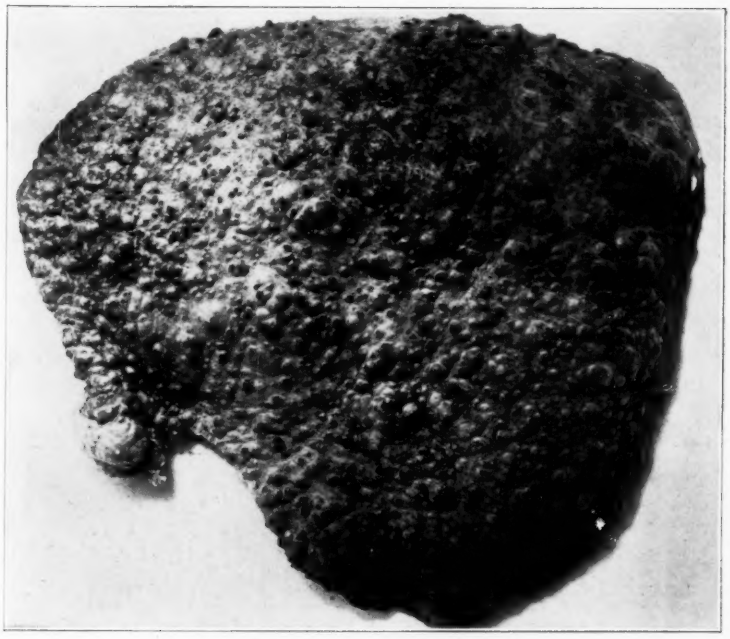

Fig. 19.—External appearance of typical hobnail liver in alcoholic cirrhosis.

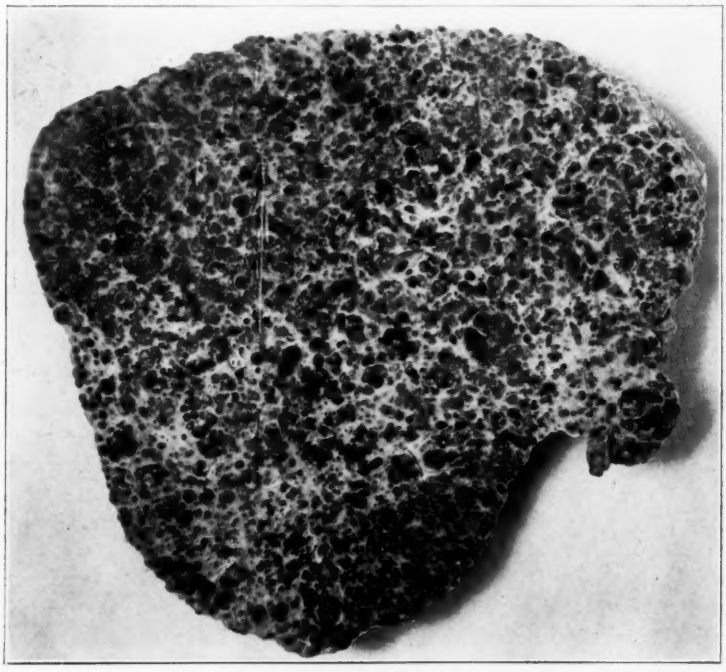

Fig. 20.—Section of liver shown in Figure 19.

hemosiderin, but the animal died not from the liver lesion but from marked deposit of hemoglobin in the tubules of the kidney. Larger doses of copper produce the same condition in rabbits. It is evidence that copper must exert a hemolytic effect on red blood corpuscles.

So far as experiments have been carried out guinea-pigs do not seem to be injured by copper.

PATHOGENESIS OF HEMOCHROMATOSIS

The cause of hemochromatosis has always been a subject of great interest. A clinical study [1] of all the cases that had come to necropsy at the Boston City Hospital from 1897 to 1923 was made to see if

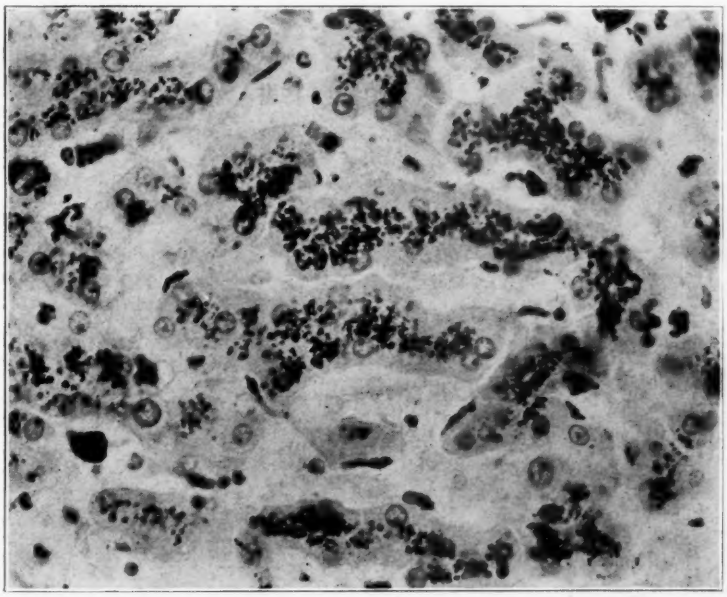

Fig. 21.—Granules of hemosiderin in liver cells in pigment cirrhosis.

they would throw any light on the etiology. It was found that the patients could be divided into three groups:

A. Those who had indulged in alcohol to excess for many years.

B. Those who had worked at an occupation involving exposure to copper.

C. Those whose histories throw no light on a possible cause.

These three groups will be discussed separately.

A. *Alcohol.*—Excessive use of alcohol has long been recognized as a possible cause of hemochromatosis. In this series of nineteen, seven

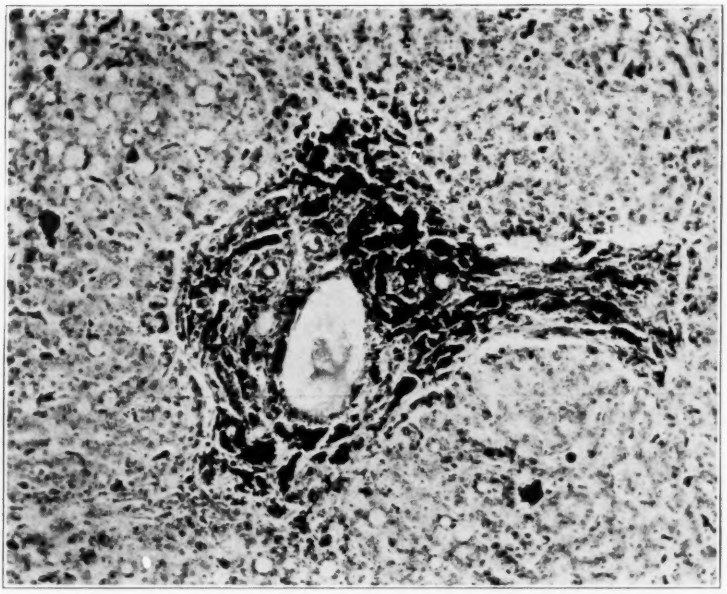

Fig. 22.—Granules of hemosiderin in liver cells and also in masses in endothelial leukocytes collected around portal vessels.

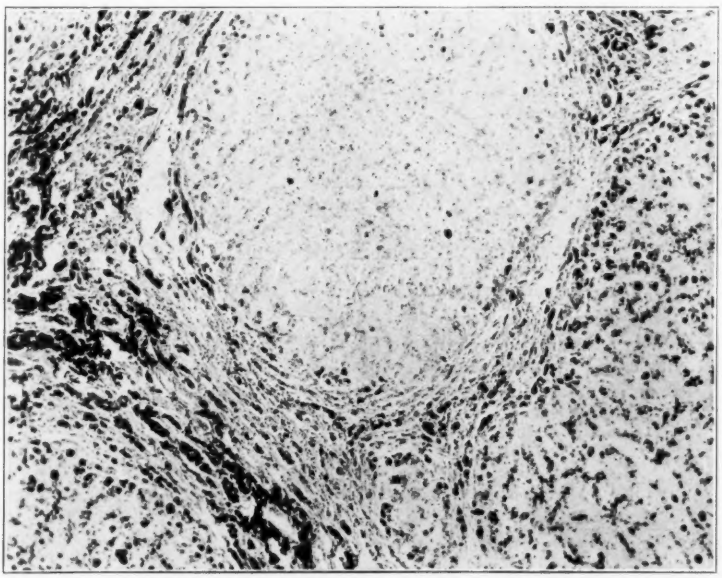

Fig. 23.—Pigment cirrhosis showing an island of regeneration in which the cells contain little pigment.

at least belong in this group. A case with the most typical alcoholic history occurred at the Peter Bent Brigham Hospital and was placed at my disposal by the surgical service. A man had been a barkeeper for four years before prohibition went into effect. Then for six years he was a bootlegger and in addition ran a private still, making corn whisky. The worm in the condenser was pure copper. He entered the hospital for hemorrhoids. The surgical intern, Dr. Harlan F. Newton, formerly an intern with me, noticed that the patient's skin was pigmented, especially over the extremities. Examination of the urine showed the presence of sugar. Sections of an excised piece of

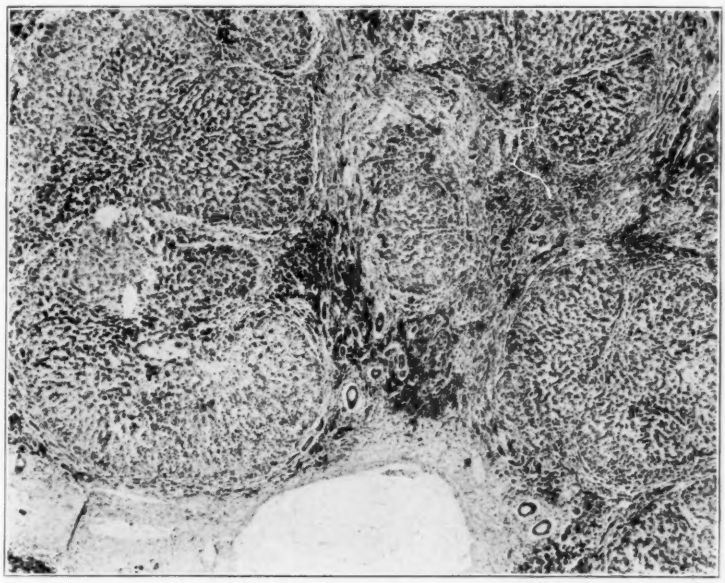

Fig. 24.—Late stage of pigment cirrhosis.

skin gave a marked iron reaction owing to a deposit of hemosiderin around the coil glands and also in fibroblasts and fat cells.

A series of eight distilled liquors examined for me by Dr. Lawrence T. Fairhall showed in seven from a trace to 10 mg. of copper to the liter. The eighth contained 185 mg. to the liter.

One of my former interns, now a surgeon, Dr. Halsey B. Loder, brought in a sample of home brew manufactured by a patient whom he had just tapped for ascites secondary to cirrhosis of the liver. The addition of a few drops of a 2 per cent solution of ferrocyanid of potassium caused a well marked reddish brown. Chemical examination showed the presence of 25.5 mg. of copper to the liter. This test is

Fig. 25.—External appearance of liver of pigment cirrhosis.

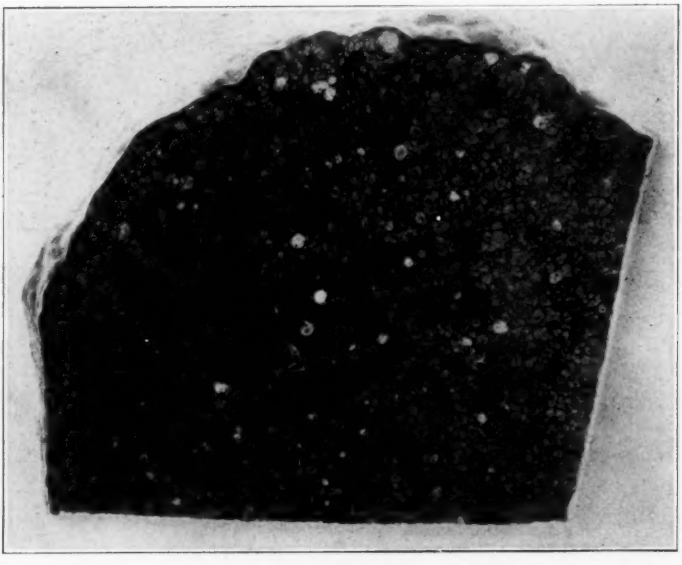

Fig. 26.—Cut surface of liver shown in Figure 25; the color of the recent islands of regeneration varies from white to gray.

not so delicate as that with potassium xanthogenate, which yields a bright yellow color, but both work perfectly satisfactorily with specimens of liquor even when they are more or less colored. Using the first method, Herman C. Lythgoe, director of the division of foods and drugs of the department of public health of Massachusetts, has tested 798 samples of "hooch" and found copper present from a trace up to 32 mg. to the liter in 10.8 per cent.

If a man were to drink a quart of whisky a day of the variety containing 185 mg. of copper to the liter (a not at all impossible feat) he would take into his system about 1 Gm. of copper a week, or 52 Gm.

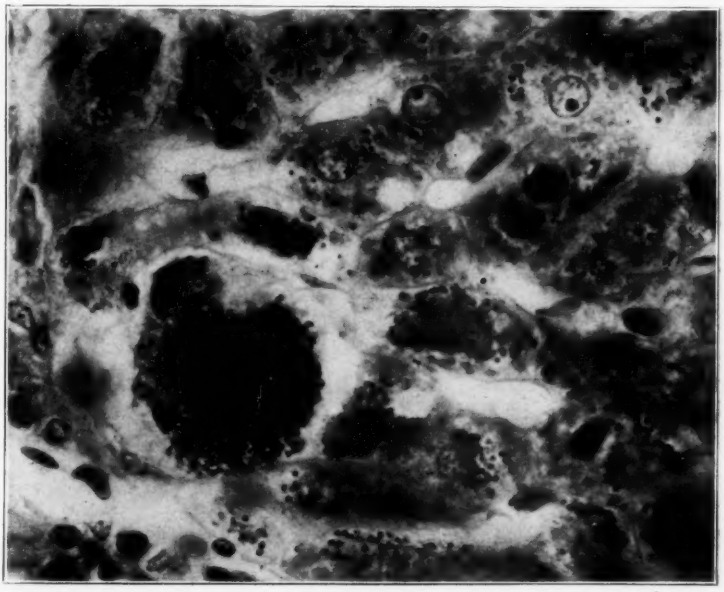

Fig. 27.—Granules of hemofuchsin in the liver cells and in endothelial leukocytes following poisoning with copper.

a year. It requires, according to all clinical evidence, at least ten and probably from fifteen to twenty-five years or more to produce the symptom complex known as hemochromatosis. In ten years he would have ingested over a pound (0.5 Kg.) of copper or 3 pounds (1.4 Kg.) of one of its salts. This amount would compare favorably, taking into consideration time and weight, with the amount of copper needed to produce pigment cirrhosis in the rabbit or sheep.

The natural inquiry is, How does the copper get into the liquor? It is easy to show that it is due to the action of organic acids in the mash distilling over with the alcohol and acting on the copper worm

of the condenser when that metal is used. A simple and quick way of testing the action of acids on copper is to shake a small amount (0.1 Gm.) of metallic copper in fine powder form in 10 cc. of a 1 per cent solution of any acid in question for fifteen minutes and then to filter. Some of the acids will color the solution bluish green in this time. The action of nitric acid is the most marked but citric acid comes close to it and then follow malic, lactic, acetic, tartaric, tannic and other acids. By precipitating all of the copper in solution by means of ferrocyanid of potassium an idea of the relative amounts dissolved by the different acids can be obtained.

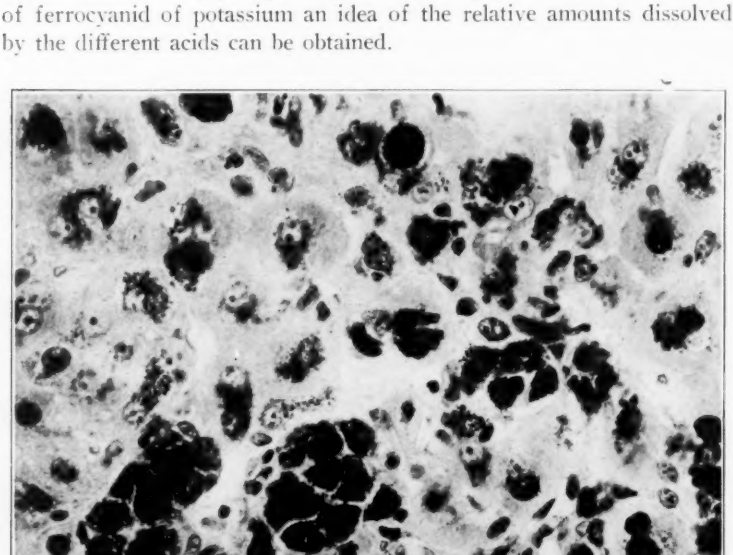

Fig. 28.—Many endothelial leukocytes containing hemofuscin granules obtained from digesting necrotic liver cells containing them.

B. *Occupation.*—Three of the nineteen patients had worked for from thirteen to thirty-eight years in foundries exposed to the dust from copper and brass. A fourth was a telephone lineman for twenty-three years, constantly handling, cutting and scraping copper wires.

The most typical illustration of the relation between occupation and disease was again furnished by the Peter Bent Brigham Hospital through the kindness of Dr. Henry A. Christian. The patient had worked for fourteen years in a shop "milling and turning copper and brass."

Three men who came to necropsy at the Boston City Hospital had worked for from six to forty-five years in brass foundries. Although no cirrhosis was present all three showed the two characteristic pigments, hemofuscin and hemosiderin, in moderate amounts in the liver and in the pancreas, and skin excised during life from two who showed slight pigmentation gave a moderate iron reaction.

The question of what would become of copper dust inhaled or swallowed led to experimental work which at first sight seemed foolish to attempt. The result of it, however, was to find that metallic copper in fine powder form injected in suspension in water or gelatin solution

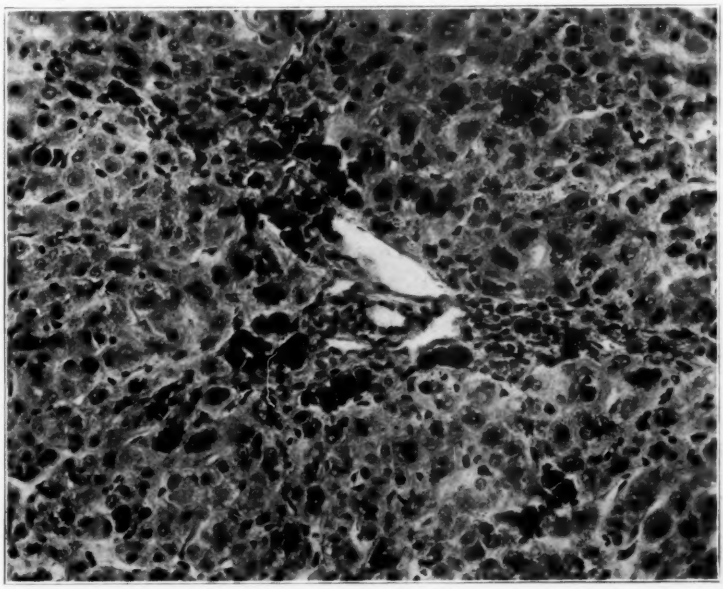

Fig. 29.—Early stage of copper cirrhosis.

was readily dissolved in the body no matter how it obtained entrance, whether through the lungs or the stomach, injected into the blood stream or subcutaneously. It seems most probable, judging from the recent work of Rous, that the copper is dissolved owing to the acid reaction of the endothelial leukocytes which quickly take up the particles of metal. When powdered metallic copper was injected into the lung the solution formed was so strong that it caused necrosis of the tissue. When injected subcutaneously the surrounding muscle fibers were killed and lime salts were deposited in them within a few days. The local inflammatory reaction was always well marked.

The general effect of the copper salt formed from the metal was the same as obtained by injecting or feeding acetate of copper. Hemofuscin was deposited in the liver and in time gave rise to pigment cirrhosis. If too much copper was administered hemoglobinuria occurred, indicating excessive destruction of the red blood corpuscles.

C. *Cause Unknown.*—In more than a third of the cases of hemochromatosis no evident source of chronic poisoning with copper was apparent. It was necessary, therefore, to consider the possibility of other sources than those connected with alcohol and occupation.

Ten cases occurring in one year gave opportunity for chemical examination of the liver and other organs for copper. In one instance

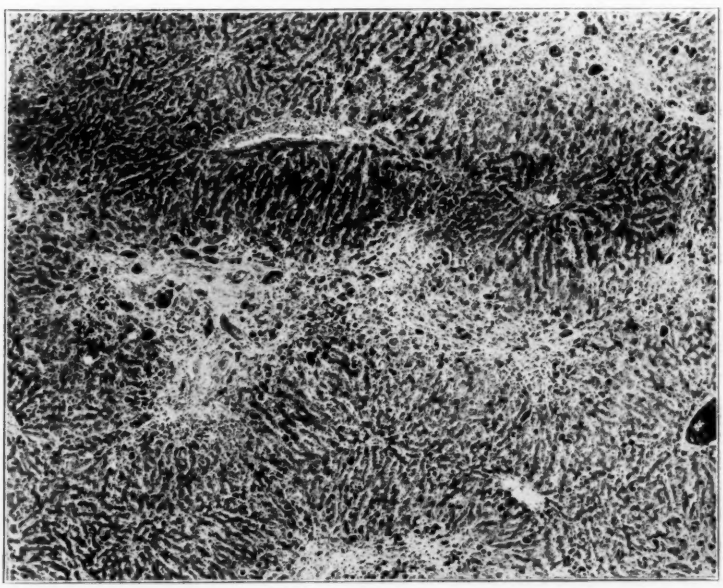

Fig. 30.—Late stage of copper cirrhosis: much sclerosis present.

as much as 20 mg. was obtained from a kilogram of tissue and less amounts in others, but a series of controls yielded results almost as high, indicating that we must be constantly exposed to the danger of ingestion of copper.

One of the patients in this group had worked for eighteen years in a cannery cooking fruits in copper kettles. Ingestion of a sufficient quantity of canned fruits containing copper might in time produce pigment cirrhosis. That is a source of poisoning to which we are all exposed. Before making any claims for this possibility, however, it would be necessary to examine chemically a series of acid fruits that

had been cooked in copper kettles to find out if they contained any copper and if so how much.

Apple butter is much used in some parts of the country and is commonly made in copper kettles. The malic acid in it readily attacks copper. This would seem, therefore, to be another source for copper poisoning. The same is true of copper tea and coffee pots, as copper is dissolved to a slight extent by the tannic acid contained in tea and coffee.

If copper powder is shaken in melted lard for fifteen minutes and then filtered out the lard will be found to be colored blue green owing to the formation of copper oleate. The other fatty acids produce the same effect.

Hess [3] has found that milk pasteurized by running over copper pipes may contain as high as 2 mg. of copper to the liter, and that this amount interferes with the action of vitamin C so that scurvy may be produced.

Cocktail shakers are usually made of copper or brass and lined with silver or tin. When the latter metals are worn or dissolved off, the acids, for example citric, in the ingredients readily attack the copper. One Boston physician informed me recently that he was now mixing cocktails in two glasses. The shakers used at soda water fountains for mixing lemonades are usually made of copper.

The brass water pipes in Brookline, where I live, are so eroded after from fifteen to eighteen years that a pin can be thrust through them almost anywhere. The amount of copper obtainable from water running through such pipes must be minute but it represents a possible though unlikely source of danger.

The examples cited here show that we are constantly exposed to the action of copper. The fact that our organs on chemical examination yield a certain amount of it does not mean that copper is a normal and necessary constituent of the body but that we cannot get away from it. This does not mean, of course, that we are all suffering from copper poisoning. It seems perfectly evident that we can handle a certain amount of the metal without danger, perhaps from 5 to 10 mg. a day, possibly more. The only visible effect may be the presence of a little hemofuscin in fibroblasts in the liver and some of the other organs where it cannot be transformed into hemosiderin and then be gradually dissolved and removed. If, however, the amount absorbed exceeds a certain definite limit the danger of pigmentation of the liver and pancreas is evident.

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3. Hess, A. F., and Weinstock, Mildred: Catalytic Action of Minute Amounts of Copper in Destruction of Antiscorbutic Vitamin in Milk, J. A. M. A. **82**:952-956 (March 22) 1924.

It is quite possible that susceptibility may play an important part. Some rabbits succumb much more quickly than others. Sheep are very sensitive while guinea-pigs seem to be almost or entirely immune.

Apparently the chief action of copper absorbed into the body is to cause hemolysis of the red blood corpuscles so that the hemoglobin is set free in the circulation. Part of the hemoglobin is eliminated through the kidneys but any excess is deposited in a changed condition as hemofuscin in the liver and later in some of the other organs.

The hemofuscin is not a compound of copper with hemoglobin but an intermediate product between hemoglobin and hemosiderin. This can be demonstrated by repeated injections of hemoglobin, obtained by the ether method from rabbit's blood, into another rabbit. In a few days hemofuscin will begin to be deposited in the liver. Hemofuscin is also formed around old hemorrhages where hemoglobin is being transformed by endothelial leukocytes and fibroblasts into hemosiderin.

CONCLUSIONS

Evidence is slowly but steadily accumulating in favor of the view that chronic poisoning with copper causes the symptom complex known under the different names of hemochromatosis, bronzed diabetes and pigment cirrhosis.

Copper is an exceedingly useful and indispensable metal in many ways but it should not be employed where it may come in contact with foods or drinks, especially if they contain acids of any sort, because it is so readily dissolved by many of them. This would exclude its use for cooking utensils of any sort, for shakers of cocktails and acid drinks, and for the worms of the condensers in stills.

Its use in pipes for drinking water and for hot water heaters is probably without danger.

PRIMARY CARCINOMA OF THE LIVER*

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In the last ten or fifteen years a voluminous literature has been accumulating with regard to the effect of chronic irritations and destructive processes on the development of cancer. The relation between normal, hyperplastic, adenomatous and cancerous changes is still extremely obscure, but it is highly probable that there is a definite connecting link. Where the boundary line between a benign and a malignant cell can be drawn, and what agencies or conditions are responsible for the change from the one to the other, are questions on which diverse opinions are held. The work of MacCarty [1] and Broders [2] on cellular pathology and on the factors influencing malignancy are important. Primary carcinoma of the liver occupies a unique position among malignant tumors, not only on account of its rarity but also from its habits of growth and spread, and from its frequent association with cirrhosis. In studying the five cases reported here we have paid particular attention to the cirrhotic element, and to its probable relationship to the accompanying malignant change.

A complete review of the literature would be impossible; for this the papers of Eggel,[3] Goldzieher and von Bókay,[4] Winternitz,[5] and others should be consulted. It will suffice to state that our knowledge really begins with the work of Sabourin [6] in 1881 and of Hanot and Gilbert [7] in 1888. Prior to that time primary carcinoma was considered much more common than secondary carcinoma and as a result the

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* Work done in the section on pathologic anatomy, Mayo Clinic.

1. MacCarty, W. C.: A Biological Conception of Neoplasia: Its Terminology and Clinical Significance, Am. J. M. Sc. **157**:657-674 (May) 1919.

2. Broders, A. C.: Cancer's Self-Control, M. J. & Record **121**:133-135 (Feb. 4) 1925.

3. Eggel, H.: Ueber das primäre Carcinom der Leber, Beitr. z. path. Anat. u. z. allg. Path. **30**:506-604, 1901.

4. Goldzieher, M., and von Bókay, Z.: Der primäre Leberkrebs, Virchows Arch. f. path. Anat. **203**:75-131, 1911.

5. Winternitz, M. C.: Primary Carcinoma of the Liver, Rep. Johns Hopkins Hosp. **17**:148-184, 1916.

6. Sabourin, Charles: Contribution à l'étude des lésions due parenchyme hépatique dans la cirrhose; essai sur l'adénome du foie, Paris, 1881, No. 39.

7. Hanot, V. C., and Gilbert, A: Etudes sur les maladies du foie: cancer (epithéliome); sarcome; mélanomes; kystes non parasitaires; angiomes, Paris, Asselin and Houzeau, 1888.

statistics were confused. Despite Virchow's warning that before a diagnosis of primary carcinoma could be made a most careful search of the eye, breast and gastro-intestinal tract should be carried out for a primary focus, series of cases were reported which were later considered entirely unacceptable. Thus of the nineteen cases of Frerichs,[8] von Paul[9] and Lancereaux,[10] von Hansemann[11] rejected all but two. He also demonstrated thoroughly that the incidence was low in comparison with that of secondary growths, by finding four cases in 258 in marked contrast to Leichtenstern's[12] seventy-two cases of primary carcinoma in 430 of both types.

In Hanot and Gilbert's series three gross types were recognized, "cancer massif," "cancer nodulaire" and "cancer avec cirrhose," with a further microscopic division into "cancer alvéolaire" and "cancer trabéculaire." Eggel, in an examination of 164 cases, came to the conclusion that this classification was unsatisfactory, especially with regard to "cancer avec cirrhose." Cirrhosis was found to be present in all types so that a separate class was not justified. He used a gross classification of massive, nodular and diffuse carcinoma, to this we adhere. Yamagiwa[13] dispensed with the rather cumbersome carcinoma adenomatosum, or "alvéolaire," and carcinoma solidum, or "trabéculaire," and substituted the term hepatoma for carcinoma of the liver cells and cholangioma for carcinoma of the bile ducts. By carcinoma of the bile ducts we mean only those arising from the smallest bile canaliculi and not those from other parts of the biliary apparatus. Although hepatoma was used originally by Sabourin to describe the condition of nodular hyperplasia, which he considered a transitional stage between adenoma and carcinoma, subsequent writers, following Yamagiwa, have appropriated it as a term for primary carcinoma of the hepatic cells. The French school as represented by Brulé[14] still adheres to the classification and conceptions of Hanot and Gilbert, but the consensus of opinion elsewhere is that Eggel's three groups are the most satisfactory.

REPORT OF CASES

CASE 1.—A man, aged 44, was admitted to the clinic, Nov. 13, 1914, complaining of pain in the lower part of the abdomen. Two weeks before admission, while he was at work, he had felt a cramplike pain around the umbilicus. His

8. Frerichs, quoted by von Hansemann, D.: Ueber den primären Krebs der Leber, Berl. klin. Wchnschr. **27**:353-356, 1890.

9. Von Paul, quoted by von Hansemann (Footnote 8).

10. Lancereaux, quoted by von Hansemann (Footnote 8).

11. Von Hansemann (Footnote 8).

12. Leichtenstern, quoted by Eggel (Footnote 3).

13. Yamagiwa, K.: Zur Kenntnis des primären parenchymatösen Leberkarzinoms ("Hepatoma"), Virchows Arch. f. path. Anat. **206**:437-467, 1911.

14. Brulé, M., in Castaigne, J.: Traité de pathologie médicale de thèrapeutique appliquée, Paris, **12**:383, 1923.

appetite was not affected, but he vomited occasionally after working. A few days before admission, he had noticed that the abdomen was swelling and that the pain, which was more severe after defecation, had shifted to the right upper quadrant. During the last year he had lost 25 pounds (11.3 Kg.) in weight.

Examination showed mild jaundice; the abdomen was tympanitic and tender below the right costal margin, but dull in the flanks owing to fluid. The spleen and the liver were both slightly enlarged, the liver extending 7 cm. below the right costal margin. On exploratory laparotomy the peritoneal cavity was found to be filled with blood stained fluid and the liver to be enlarged and nodular. A diagnosis of carcinoma of the liver was made and the abdomen was closed without further procedure. The patient gradually became weaker and died on the fifth day.

Necropsy was performed fourteen hours after death. Cirrhosis of the liver, primary carcinoma of the liver, chronic splenitis, ascites, slight bronzing of the skin, chronic catarrhal cholecystitis and dilatation of the bile ducts, chronic congestion of the abdominal viscera, myocardial degeneration and pulmonary edema were found.

The skin and mucous membranes were slightly yellow and the face and neck were dark and bronzed. There were several hundred cubic centimeters of blood tinged fluid in the peritoneal cavity. The spleen was enlarged, measuring 18 by 12 cm. The pancreas was firmer than normal, the head being slightly enlarged. The lymph nodes at the hilum of the liver were enlarged and soft. The gallbladder was dilated and contained about 15 cc. of thick, mucoid bile, but there were no lesions of the mucosa. The extrahepatic bile ducts were dilated (Grade 3) but patent throughout. The portal and the mesenteric vessels were dilated. The liver was enlarged and extended 7 cm. below the costal margin. The whole surface was coarsely nodular, the largest nodule being situated in the right lobe. The nodules varied in diameter from a few millimeters to 5 cm. The capsule was thickened, opaque and yellowish white without adhesions to the surrounding structures. On section the entire right lobe appeared to be replaced by nodules, each one being surrounded by a thin fibrous capsule. Many of them were grouped in masses and were surrounded by a thicker capsule of compressed liver tissue. In the left lobe they tended to be more discrete. Most of them were cream colored, but the color varied from light brown to greenish red. The consistency near the periphery was firm, but in the center it was soft and putty-like. The remainder was dark green, compressed, scarred and coarsely granular. The fibrous tissue trabeculae produced a network arrangement throughout the entire section. A main branch of the portal vein was completely filled with tumorous tissue resembling the larger nodules, and there were a few small blood clots around the mass, adherent to the main wall. A few of the smaller portal branches appeared to be plugged with the same tissue, but the hepatic veins were free.

Sections for histologic examination were taken from various portions of the liver. The normal architecture was in many places profoundly altered by the pressure of large and small nodules scattered throughout so that sections from any portion must necessarily include these areas. The liver tissue was everywhere involved in a marked cirrhotic process, irregularly distributed, but most abundant in the region of the periportal spaces. Here and there large and small bands of fibrous tissue extending from the periportal spaces surrounded areas of liver tissue, which had for the most part lost its radiate arrangement and central veins. In other situations there were huge scars altogether devoid of liver cells. The fibrosis was more pronounced in sections in which the tumor compressed the cells. The connective tissue in the periportal spaces was rather more dense around the vessels than around the bile ducts, and was infiltrated with numerous small tortuous bile ducts, while lymphocytes, diffusely scattered in some areas and massed together in others, formed a prominent feature. They did not, however, extend into the hepatic sinusoids. The

nodules so enclosed appeared adenomatous, with the cells at the periphery clean-cut, convex and more intensely stained with hematoxylin than the central cells. It was evident that hyperplastic processes were in progress in this situation. Fatty degeneration, evidenced by vacuolation and coarse granulation, was more marked in the center of these areas. Inspissated bile was present in the bile canaliculi, and the cells themselves contained bile pigment. It was particularly well marked in the connective tissue spaces. The tumorous nodules consisted of large masses of polygonal, rectangular and oval cells with a more or less trabecular arrangement. Throughout their substance were bands of connective tissue well shown by the van Gieson stain, forming a network in which the cell groups lay. For the most part, the cells lay within blood vessels which could be recognized by their intimal and muscular layers. The expansion of these vessels had produced atrophy and destruction of the surrounding liver cells. Many areas showed necrosis of the walls of the blood vessels with invasion of the hepatic parenchyma by tumor cells. Although at first sight there appeared to be a transition between normal and abnormal cells in these situations, a close examination failed to reveal any such change, and a definite demarcation between the two was always apparent. The abnormal cells varied greatly in size and appearance, but for the most part they bore a general relationship to the normal liver cell. The cytoplasm was rather granular and showed fatty changes, but the nuclei stained deeply and were hyperchromatic and often pyknotic. Mitosis was frequent and giant cell forms were abundant. Bile pigment could not be distinguished within the cells, but it appeared in the intercellular spaces and around the periphery in the connective tissue. Where the tumorous emboli had retracted from the vessel wall, the endothelium adhered to the surface. The cells extended into small branches of the portal vessels and then expanded into nodular masses filling every crevice of the vascular spaces. The malignant tissue was well preserved and distinguishable from the normal parenchyma by its more intense staining reaction, prominent capillary stroma, encapsulation by extremely dense fibrous tissue, and branchlike character where tumor thrombosis had occurred. Although grossly the hilum nodes were enlarged and soft, no tumorous tissue was discovered microscopically. This case was undoubtedly a primary carcinoma of the liver cells or hepatoma of the diffuse form.

Case 2.—A man, aged 63, was admitted to the clinic, June 23, 1923, complaining of swelling of the abdomen and pain at the right costal margin. He had suffered from gastric disturbances periodically for three years, but had not been incapacitated until four months before his examination when he began to have frequent attacks of severe pain in the right upper quadrant associated with bloating, nausea and more recently vomiting of dark brown material. One month later the pain became more severe, and a tender mass could be palpated below the right costal margin extending across the epigastrium just above the umbilicus. The patient had lost 15 pounds (6.8 Kg.) during the previous four months. He had noticed an occasional dark stool.

On examination the skin and mucous membranes were slightly jaundiced, and there was slight bronzing of the face and neck. The abdomen was distended and there was shifting dulness in both flanks. A mass was palpable across the epigastrium. The legs were slightly edematous. The blood was chemically normal.

Necropsy, performed three and one-half hours after death, revealed primary carcinoma of the liver with extension to the portal and hepatic veins, inferior vena cava, right auricle and branches of the pulmonary artery, metastasis to the lungs, cirrhosis of the liver with adenomas, ascites, old healed pulmonary tuberculosis with tuberculous pleuritis, and splenitis.

The skin was moderately icteric and slightly bronzed over the face and neck. The abdomen was distended. The peritoneal cavity contained approximately 3,000 cc. of blood tinged fluid. The lower margin of the liver extended

8 cm. below the costal margin. The gallbladder was normal. Numerous small, yellowish white nodules, measuring from 3 mm. to 1 cm. in diameter, projected through the pleura of both lungs. On section these were yellowish white and seminecrotic. The main branches of the pulmonary artery were free from emboli, but masses of yellowish white, tumorous tissue extended from the right auricle down into the inferior vena cava.

The liver was extremely irregular and mostly replaced by numerous yellowish white nodules which were plainly seen beneath its capsule. The liver tissue had been displaced between the capsule and the nodules. On section the greatest portion of the clay colored tissue was seen to consist of one mass, which involved approximately four-fifths of the right lobe. It was quite homogeneous, but the outer, lower and central portions near the hilum of the liver were divided by compressed fibrous tissue and remnants of liver into small, irregular, pasty nodules from 1 to 8 mm. in diameter. In the upper part of the largest mass was a circumscribed area, measuring 4 cm. in diameter, which consisted of reddish brown necrotic material studded by numerous small hemorrhages. On the left side near the hilum the tumor had invaded the portal vein and occluded it completely. This plug filled all the branches of the right lobe of the liver but only extended for a short distance into the branches of the left lobe. The inferior vena cava was likewise occluded by a mass which projected into it from the hepatic veins below. The lower one fifth of the right lobe was almost free from nodules, and was markedly compressed and congested. The liver tissue here was dark brown and divided by prominent irregular bands of fibrous tissue, which gave it a coarsely granular appearance. The left lobe of the liver was small and had the same granular structure.

Representative sections were taken from various portions of the liver for microscopic examination. Sections from the left lobe and the tip of the right showed extensive cirrhosis in progress. The capsule was much thickened and beneath it were dense collections of lymphocytes extending among the columns of the liver. Bands of fibrous tissue extended to the nearest portal spaces and from these further fibrous septums marked off well defined nodules of liver tissue. Wherever the fibrous tissue was present, lymphocytes were prominent, this being especially notable in the region of the portal spaces. Here, too, there were numerous tortuous bile ducts and endothelium lined spaces which were probably hepatic veins. Small groups of liver cells had been buried in the depths of the connective tissue, and had regenerated to form around adenomatous nodules. The liver tissue itself was infiltrated with lymphocytes, while destruction of the liver cells, atrophy and congestion were prominent features. As has been noted, the liver tissue was for the most part marked into large nodules consisting of many hepatic lobules. The normal radiate arrangement was considerably altered and hepatic central veins were uncommon. Around the edges of the nodules, large, finely granular, deep-staining cells with large nuclei indicated active new cell formation. At other points degeneration was proceeding. This is the hyperplastic adenomatous type of portal cirrhosis. The malignant areas showed dense masses of polygonal, triangular and large oval cells arranged in trabeculae and columns. These cells could be easily recognized by their great irregularity, deep-staining properties and diverse nuclear forms. Mitotic figures were common and multinucleated cells were often seen. Considerable fibrous tissue had developed between the cell columns and a capillary stroma was prominent. In the centers of the nodules degenerative changes were visible, while at the periphery the structure was better preserved. Although most of the nodules had a dense fibrous tissue capsule in which many atrophic liver cells and bile ducts were present, there had been direct invasion of the hepatic substance. It is in these situations that transitional forms are searched for, but it always seems possible to distinguish quite well between normal and malignant cells; that is, the process is an infiltrative one. Many branches of the portal veins were plugged with thrombi of the tumorous cells.

From the cell type and its arrangement there was no doubt that the tumor was a hepatoma or primary carcinoma of the liver cells. In the metastatic nodule from the right auricle, pulmonary artery and lungs, the same type of cell with the same arrangement was seen. In the lungs the tumors were situated within the pulmonary artery and its smaller branches, dilating the lumen and producing marked thinning of the walls.

Case 3.—A man, aged 54, was admitted to the clinic, March 20, 1924, complaining of stomach trouble. Eighteen months previously, after a severe headache, he had vomited a small amount of blood, and a few days later had noticed a hard lump in the left upper quadrant of the abdomen. A second hemorrhage occurred one month later, more severe than the first, leaving him markedly jaundiced, but the jaundice gradually subsided. Fats and coarse vegetables induced epigastric distress with bloating and belching. His weight was well maintained, but he had gradually lost strength.

On examination the patient was found to be well nourished, slightly anemic, with edema of the feet and moderate distention of the abdomen. The liver and spleen were tender and enlarged (Grade 2). The hemoglobin was 44 per cent; erythrocytes numbered 37,300, leukocytes 6,600, and the blood platelets 124,000. The fragility test indicated an increase in red cell resistance. The coagulation time was five minutes, and the prothrombin time ten minutes. A diagnosis of Banti's disease was made. On exploratory operation the condition in the liver was observed and the abdomen closed without further procedure. The patient gradually became weaker, and died twelve hours later.

Necropsy was performed nine hours after death. Primary massive carcinoma of the liver involving the right lobe; ascites; esophageal varices; left hydronephrosis (Grade 4); old healed mitral and aortic endocarditis with hypertrophy of the heart (490 Gm.), and fatty and fibrous changes of the myocardium were found.

The abdomen was moderately distended. The peritoneal cavity contained about 1,500 cc. of fluid with no evidence of peritonitis. The gallbladder contained 25 cc. of thick mucoid bile; the mucosa was normal. The extrahepatic bile ducts, pancreas, stomach and duodenum appeared normal. There were a few varices of the esophagus and a small amount of free blood in the ileum. The liver weighed 2,810 Gm. and the right lobe extended 6 cm. below the costal margin in the right clavicular line, forming a massive tumor with a coarsely nodular surface. The left lobe was finely granular and had a thickened opaque capsule. Situated on the inferior and the posterior surface of the right lobe was a spherical mass which was slightly elastic to pressure and measured 15 by 13 cm. On section the entire right lobe except a small margin near the hilum was replaced by a greenish white tumor, putty-like in consistency, and surrounded by a firm white capsule from 2 to 3 mm. in thickness. Delicate fibrous trabeculae from the capsule divided the tumor into small, irregular masses, and in the meshes of this network lay the pastelike tumorous tissue. In the superior portion was a cavity measuring 3 by 3.5 cm., containing clotted blood, with a similar but smaller cavity just below and to the right. In the adjacent liver tissue near the left lobe were many smaller encapsulated nodules similar in structure to the main mass and apparently metastasizing from it. Grossly it was impossible to determine whether any branches of the portal or hepatic veins were occluded by the tumorous tissue. In the left lobe were bands of connective tissue enclosing small white nodules, giving it the typical cirrhotic character. It was practically free from nodules of the appearance and consistency of those in the right lobe.

Microscopic sections from various parts of the right lobe showed a cirrhotic process generally distributed throughout its whole extent, but more marked in the region of the tumor. The bands of connective tissue surrounded large areas of parenchyma of the liver. The capsule was thickened and from it dense bands of fibrous tissue extended into the hepatic substance marking off circular

areas consisting of numerous individual lobules. The connective tissue contained many tortuous bile canaliculi, most marked at the periphery of these conglomerate masses. Not only was this area infiltrated with large numbers of lymphocytes, but the whole structure of the liver was invaded more or less diffusely and where they were assembled in groups, strands of connective tissue extended irregularly among the hepatic columns. The nodules of liver cells contained a few central sinusoids, most of them being irregularly disposed with regard to the connective tissue, and indeed many appeared incorporated with it. Congestion of the sinusoids was particularly prominent and fatty degeneration was common. The cells at the periphery of the adenomatous masses showed a faintly granular cytoplasm, staining more evenly and deeply than the central cells; the nuclei were clear cut, occasionally double, and with one or two mitotic figures. Bile staining was prominent throughout the section. The tumors consisted of dense masses of more or less polygonal cells having a definite trabecular arrangement well preserved throughout. They stained irregularly and the cytoplasm was vacuolated and coarsely granular. The nuclei varied greatly in size, were pyknotic and hyperchromatic, taking the hematoxylin stain more intensely than the nuclei of the normal liver cell. In many situations the cells had a rosette formation, but no lumen was visible. Multinucleated giant cells and mitotic figures were numerous. Fibrous tissue was not a marked feature among the trabeculae but as the periphery was reached the van Gieson stain revealed it to be well developed. A capillary stroma, however, was prominent throughout. Around each mass were extremely dense bands of homogeneous connective tissue containing a few lymphocytes and vessels filled with columns of tumorous cells. Even after careful search no transitional stages between liver and malignant cells could be demonstrated, nor was any intracellular bile pigment found. The nodes at the hilum showed no metastatic deposits. This tumor was a primary liver cell carcinoma or hepatoma of the massive form.

Case 4.—A man, aged 48, was admitted to the clinic, May 28, 1925, complaining of pain in the upper abdomen, nausea, vomiting and belching, with loss of weight and strength. Two years prior to admission, he began to have epigastric pain, which persisted and gradually increased up to the present time, associated with bloating and belching after meals. Twenty-two months after the onset he had daily attacks of nausea and vomiting which continued for three or four weeks, nausea alone remaining after the vomiting subsided. He was forced to stop work. He had lost 20 pounds (9 Kg.) in the last two months.

The patient was slightly emaciated, with dark skin and yellowish conjunctivae. The abdomen was slightly distended and tympanitic. A palpable and tender mass, extending 7.5 cm. below the right costal margin and over on to the left side, was found in the upper part of the abdomen. It was smooth on the right side but markedly nodular on the left. The whole tumor moved with respiration. A diagnosis of malignant disease of the liver was made, and the patient was admitted to the hospital for observation, where he gradually became weaker and died ten days later.

Necropsy was performed two and one-half hours after death. Primary carcinoma of the liver with invasion of the sixth thoracic vertebra and compression myelitis, gangrenous cystitis (cord bladder) and pyelonephritis, pneumoconiosis 3, old healed pulmonary tuberculosis with tuberculous pleuritis and arteriosclerosis 1 were found.

The skin was universally dark brown and the conjunctivae icteric. There was about 200 cc. of clear yellow fluid in the peritoneal cavity. The spleen weighed 255 Gm. and presented a moderate perisplenitis. Both kidneys showed extensive pyelonephritis, and there was a gangrenous cystitis of the urinary bladder, the mucous membrane being 1 cm. thick, dark brown and necrotic. The pancreas and gastro-intestinal tract were normal. The gallbladder was moderately distended with 80 cc. of black mucoid bile, but there were no lesions

of the mucosa. The common, cystic and hepatic ducts appeared normal. The liver was enlarged, extending 7.5 cm. below the right costal margin. It weighed 3,100 gm. The surfaces of the left lobe and approximately the left half of the right lobe were coarsely nodular and slightly greenish yellow. The remaining surface of the right lobe was studded with minute greenish brown nodules shining through the capsule, which was finely granular throughout. On section the whole of the left lobe was found to be replaced by multiple nodular tumors varying in diameter from 1 mm. to 0.5 cm. Each was surrounded by dense bands of grayish fibrous tissue. The branches of the portal vein were occluded by masses of soft velvety green material interspersed with small blood clots. Toward the middle portion of the liver the nodules became more discrete, but even here the portal vessels were seen to be plugged with the same tissue. The portal vein at the hilum was occluded by a red and white striated thrombus in which small masses of yellow material could be recognized. In the right lobe there were many discrete nodules of similar appearance pushing aside and compressing the hepatic tissue. Here the liver was coarsely granular with small greenish areas of tissue surrounded by bands of fibrous tissue. It had the typical appearance of a well advanced cirrhosis.

Microscopic sections were taken from various portions of the liver and from the metastatic tumors. In the areas where tumorous tissue was not grossly evident, an extremely active cirrhotic process was in progress. The periportal spaces were surrounded by dense masses of connective tissue, with fibrous septums extending into the parenchyma. These marked off large areas of hepatic tissue forming circular nodules. The abundance of lymphocytes was striking. They were in great masses between the connective tissue and the liver cells and extended out among the acini of the parenchyma; the sinusoids contained great numbers. Wherever these lymphocytes were collected, destruction of liver cells was in active progress, and a replacement fibrosis was occurring. At the same time hyperplastic and regenerative changes were taking place in the liver cells in these areas, and columns of clear cut, convex and more deeply stained cells crowded into every corner and crevice of the connective tissue. The adenomatous nature of the change was evidenced also by the shape of the nodules, which consisted of many individual hepatic lobules with few central veins. The radiate arrangement of the acini had disappeared, and the newly formed nodules bore no relation to the original hepatic structure. Tortuous bile canaliculi were actively growing wherever fibrosis had occurred, although no liver cell regeneration from them could be made out. The area of malignant cells consisted of dense masses of cuboidal cells, having a more or less tubular arrangement, and set in extremely compact fibrous tissue. The cells themselves varied from cuboidal to columnar and formed columns and tubules with small lumina, frequently plugged with bile thrombi. Bile was not visible within the cells but there was no doubt that it frequently occurred in the tubular spaces. The cells themselves stained more deeply than the liver cells and the nuclei were round, oval or distorted, and were composed of granular protoplasm. Mitotic figures were not frequent although there was a tendency to pyknosis. In their staining reactions and general shape they strongly resembled the cells of the bile canaliculi. There was a poorly developed capillary stroma through the tumor, but a large amount of connective tissue. This fibrous tissue was homogeneous, and although it contained few lymphocytes, bile canaliculi were common. In one situation the malignant tubules diffusely invaded the fibrous tissue and infiltrated the hepatic sinusoids. Mixed with them were large numbers of obviously tortuous bile ducts which seemed too numerous and too hyperplastic to be regarded as entirely normal. We are inclined to think, however, that these are not transitional forms. Some of the spaces in which the tumor lay were lined by endothelium, and where retraction from the wall had taken place the endothelium followed the tumor. It seemed, therefore, that these spaces were originally of vascular origin. From the cell

type and its relation to the bile canaliculi this tumor should be classified as a cholangioma or primary carcinoma of the small bile ducts occurring in a cirrhotic liver.

CASE 5.—A man, aged 60, was admitted to the clinic, Aug. 8, 1925, complaining of a sharp pain of three months' duration in the right upper quadrant. The pain was troublesome during the day but not at night, and bore no relation to food. There was neither nausea nor vomiting. Of late his bowels had moved from four to six times a day, and occasionally he had passed a little blood. He had lost about 10 pounds (4.5 Kg.) in weight.

On examination the abdomen was tense and tender, particularly from the umbilicus to the xiphoid. A mass was found in the right upper quadrant. There were also signs of fluid in the flanks. A test of hepatic function showed dye retention 3; serum bilirubin was 1.6 mg. for each hundred cubic centimeters of blood. The hemoglobin was 57 per cent. An exploratory operation was performed, and extensive cirrhosis of the liver with ascites was found. The liver was about twice the normal size, and on account of the markedly nodular condition malignancy was suspected. The abdomen was closed without further procedure. The patient gradually became weaker and died on the third day.

Necropsy was performed two hours after death. The findings were cirrhosis of the liver with adenoma formation and malignant change, varices of the veins of Sappey and of Ritzius, and of the esophagus, with hemorrhage from the first, plugging of the branches of the portal vein by tumorous tissue, splenomegaly, partial collapse of the lower lobes of both lungs, jaundice 1, multiple retention cysts of both kidneys, old healed tuberculous pleuritis and lymphadenitis, healing gastric ulcer at the pylorus, melanosis of the sigmoid and pseudomelanosis of the cecum, emaciation 3, and arteriosclerosis 1.

The skin was slightly jaundiced. The peritoneal cavity contained about 1,500 cc. of fluid, but there were no signs of peritonitis. Between the liver and the diaphragm, especially over the right lobe, were numerous dense adhesions which were broken down with difficulty. The spleen weighed 405 Gm. and presented a smooth, slaty blue capsule with slight fibrohyaline changes. On section it was dark red with well marked fibrous change in the trabeculae. The gallbladder contained 15 cc. of thick bile and presented a few papillomas of the mucosa. The common and hepatic ducts were patent. The liver weighed 2,645 Gm. The surface was extremely nodular; the nodules varied in diameter from a few millimeters to 10 cm. The largest projected from the right lobe and was densely adherent to the peritoneum covering the diaphragm. These masses were brownish yellow but the intervening tissue, which consisted of finer granulations, were bluish gray, as seen in cirrhosis. The masses were soft, semifluctuant, and in one situation where the capsule had ruptured a yellowish pasty mass was expressed. On section the liver appeared variegated, owing to the presence of multiple globular tumors scattered throughout the substance. The largest, measuring 11 cm. in diameter, projected from the right lobe and occupied almost its whole extent. In general, they were fairly sharply defined from the hepatic tissue, which was atrophied and compressed to form a definite capsule for each nodule. The color varied from yellow to light reddish pink, although in many places green predominated, especially in the larger masses. Fibrous trabeculae extended through the substance of the nodules, forming a network, in the meshes of which was embedded soft tumorous tissue of pastelike consistency. Degenerative changes and hemorrhages were common in the larger nodules; the smaller ones were firmer and of a more uniform yellow. The intervening tissue had a somewhat distinctive appearance. Here and there were small velvety brownish nodules, much firmer and more circumscribed, and surrounded by definite bands of connective tissue, giving the whole a surface like leather to the touch, and offering considerable resistance to the knife. The portal vein at the hilum was completely occluded by a thrombus of fairly recent date, but the medium sized veins were filled with soft yellow material resembling that seen in the nodules. This condition seemed

to extend out into the smallest recognizable branches of the portal system. There was no extrahepatic metastasis.

Microscopic representative sections were taken from areas throughout the liver. Those from the finely granular portion showed large nodules of apparently normal liver cells surrounded by bands of dense connective tissue. The capsule was thickened and from it extended the fibrous arches which surrounded the hyperplastic nodules. Lymphocytes were extremely numerous beneath the capsule and along these septums. In the periportal spaces, there was a great deal of scarring, the thickening being most marked in the region of the portal vessels. Collections of lymphocytes and many tortuous bile ducts were seen. Small nodules of hepatic tissue had become separated from the parent mass and remained stranded in the depths of the scars. There were no single liver cells or fragments of acini in these septums such as are seen in the intercellular type of cirrhosis, for all nodules showed definite hyperplastic processes, and single cells and portions of acini had become adenomatous masses. The remaining hepatic tissue consisted of large nodules made up of many small lobules with eccentrically placed and frequently absent central veins, where the fibrous bands had extended into the lobule and incorporated the veins in the scarring process. The radiate arrangement of the acini was profoundly altered, especially at the periphery where the cells were larger, more convex, and had the appearance of being newly formed. Fatty degeneration was not a marked feature, although there was considerable congestion of the sinusoids which also contained a good many lymphocytes. Near the malignant masses and incorporated in their capsular walls, were elongated groups of liver cells surrounded by dense fibrous tissue. They were compressed, atrophic and infiltrated with lymphocytes. Here actual necrosis of the cells had taken place and some hyalinization seemed to be occurring. Bile canaliculi containing many bile thrombi appeared in this situation. Within the tumorous capsule the cells lay in masses, chiefly arranged in columns and trabeculae. There was a small amount of capillary stroma between these budlike processes, but it was not a marked feature. Trabeculation of the tumorous cells was more common at the periphery, but toward the center a good deal of dissociation had taken place owing to necrosis. Here the individual units could be recognized as polygonal, triangular and oval cells. They varied greatly in size, the larger being multinucleated and the smaller showing a single, small, fairly deeply staining nucleus. Mitotic figures were common and all the various stages of karyokinesis could be easily followed. In the nodules in which necrosis was not a marked feature, a rosette formation was recognized with a tiny central space in this arrangement. The vessels were plugged with tumorous tissue, and in many situations an expansion of the walls occurred, causing compression and atrophy of the surrounding liver cells. A small amount of bile pigment occurred in the malignant cells themselves, although it was more frequently seen in the connective tissue at the periphery. Despite careful search, transitional forms between the liver and the cancer cells were not recognized. From the cell type and its arrangement the tumor was a hepatoma or primary carcinoma of the liver cells.

The foregoing five cases were observed in 5,976 cases of necropsy, about 0.08 per cent. During the same period, 220 cases of secondary carcinoma of the liver and 127 cases of portal cirrhosis were observed. Thus, primary carcinoma of the liver occurred once in every forty-four cases in which there was metastatic growth, and was associated with 3.9 per cent of cases of cirrhosis.

The sex incidence in cases of primary carcinoma of the liver runs parallel to that in cases of cirrhosis, that is, males predominate. In

TABLE 1.—*Findings in Five Cases of Primary Carcinoma (Modified from Eggel and as Used by Karsner)*

Case	Age, Sex*	Type of Carcinoma	Origin	Cirrhosis Type	Changes in Liver			Extrahepatic Metastasis	Duration	Jaundice	Ascites	Edema	Spleen	Liver
					Cells	Bile Ducts	Vessels							
1	44 ♂	Trabecular hepatoma of diffuse form	Liver cells, right lobe	Marked, portal	Liver cell type, frequent mitosis, little necrosis	Proliferative, interacinous	Marked tumor thrombosis	None	19 days	Slight	Marked	None	Enlarged and palpable	Enlarged and palpable
2	63 ♂	Trabecular hepatoma of massive form	Liver cells, median portion	Moderate, portal	Liver cell type, frequent mitosis	Proliferative, interacinous	Marked tumor thrombosis	Inferior vena cava and right auricle; pulmonary artery and lungs	3 years 4 months	Moderate	Marked; 3,000 cc.	Slight	Normal	Enlarged and palpable
3	54 ♂	Trabecular hepatoma of massive form	Liver cells, right lobe	Moderate, portal	Liver cell type, frequent mitosis	Markedly proliferative, interacinous	Slight tumor thrombosis	None	18 months	Slight	Marked; 2,500 cc.	Marked	Enlarged and palpable	Enlarged (2,810 Gm.), palpable
4	48 ♂	Adenocarcinoma, alveolar; cholangioma of diffuse type	Bile duct, left lobe	Marked, portal	Low columnar cuboidal-like bile ducts	Proliferative, interacinous	Marked tumor thrombosis	Spine with compression myelitis	2 years 2 months	Moderate	Slight; 200 cc.	None	225 Gm.	Enlarged (3,110 Gm.), palpable
5	60 ♂	Trabecular hepatoma of nodular type	Liver cells, right lobe	Moderate, portal	Liver cell type, frequent mitosis; necrosis marked	Proliferative, interacinous	Marked tumor thrombosis	None	3 months	Slight	Slight; 150 cc.	Slight	405 Gm.	Enlarged (2,645 Gm.), palpable

* In this table ♂ indicates male.

TABLE 2.—*Incidence as Determined by Previous Authors*

Author	Cases	Necropsies	Necropsy, per Cent	Cirrhosis, per Cent	
				Hepatoma	Cholangioma
Eggel	164	?	?	86.4	62.5
Goldzieher and von Bokay	18	6,000	0.3	100	42.8
Yamagiwa	42	?	?	74.75	46.7
Karsner [15]	9	5	?	100	100
Winternitz	3	3,700	0.08	100	100
Hale-White [16]	25	18,500	0.13	?	?
Fried [17]	4	1,200	0.33	100	100
Clawson and Cabot [18]	1	5,100	0.05	100	...
Von Glahn and Lamb [19]	6	1,800	0.33	100	100
Mayo Clinic	5	5,976	0.08	100	100

Thus, sixty-two cases occurred in 42,276 necropsies, or 0.14 per cent. The incidence of cirrhosis will be discussed later.

our five cases the patients were males, while in nineteen cases reported since 1922 by Torland,[20] Clawson and Cabot, Helvestine,[21] Williamson,[22] von Glahn and Lamb, Fried, Friedenwald and Fried,[23] Lichty and Richey [24] and Jaffé,[25] all but four were males. In thirty-two cases reported by von Heukelom,[26] thirty-one were males. In Yamagiwa's series the female sex is fairly well represented. The condition usually occurs after the fortieth year, although many cases have been reported as occurring in children. Philipp [27] reported twelve genuine cases of this kind, and Dansie,[28] twenty-five patients under 2½ years. Griffith [29]

15. Karsner, H. T.: A Clinicopathological Study of Primary Carcinoma of the Liver, Arch. Int. Med. **8**:238-261 (Aug.) 1911.

16. Hale-White, W.: Primary Carcinoma of the Liver, in Allbutt, T. C., and Rolleston, H. D.: System of Medicine, Ed. 2, London, Macmillan Company, 1908, **4**:215-219.

17. Fried, B. M.: Primary Carcinoma of the Liver, Am. J. M. Sc. **168**:241-267 (Aug.) 1924.

18. Clawson, B. J., and Cabot, V. S.: Primary Carcinoma of the Liver, J. A. M. A. **80**:909-910 (March 31) 1923.

19. Von Glahn, W. C., and Lamb, A. R.: Primary Carcinoma of the Liver, M. Clin. N. Amer. **8**:29-53 (July) 1924.

20. Torland, T.: Primary Carcinoma of the Liver, Northwest Med. **21**:253-255 (Aug.) 1922.

21. Helvestine, Frank, Jr.: Primary Cancer of the Liver, J. Cancer Research **7**:209-227, 1922.

22. Williamson, C. S.: Primary Carcinoma of the Liver, M. Clin. N. Amer. **8**:453-464 (Sept.) 1924.

23. Friedenwald, J., and Fried, H.: Primary Cancer of the Liver, Am. J. M. Sc. **168**:875-882 (Dec.) 1924.

24. Lichty, J. A., and Richey, D. G.: On Primary Carcinoma of the Liver: Report of Three Instances, Ann. Clin. Med. **1**:165-169, 1922.

25. Jaffé, R. H.: Sarcoma and Carcinoma of the Liver Following Cirrhosis, Arch. Int. Med. **33**:330-342 (March) 1924.

26. Von Heukelom, S.: Das Adeno-Carcinom der Leber mit Cirrhose, Beitr. z. path. Anat. u. z. allg. Path. **16**:341-387, 1894.

27. Philipp, P. W.: Ueber Krebsbildungen in Kindesalter, Ztschr. f. Krebsforsch. **5**:326-416, 1907.

28. Dansie, C. B.: Primary Malignant Growth of the Liver in Infants, with Report of a Recent Case, Lancet **2**:228-229 (July 29) 1922.

29. Griffith, J. P. C.: Primary Carcinoma of the Liver in Infancy and Childhood, Am. J. M. Sc. **155**:79-85 (Jan.) 1918.

recorded fifty-seven cases occurring in children under 16 years of age. The ages in our series were between 44 and 63, while the average age in our compiled series was 61½ years, none occurring in patients under 14. From the point of view of age incidence, the cases are divided into those occurring in infancy and those occurring in adult life, past the age of 40.[30] It is noteworthy that the congenital cases are rarely combined with cirrhosis and are to be regarded as instances of congenital maladjustment of groups of liver cells.[31]

The clinical diagnosis in the adult form is usually that of cirrhosis of the liver. The cases fall into two main groups, those in which there is a long indefinite history extending up to three years, with a sudden exacerbation and a termination within four months, and those in which there is a rapid progressive course, lasting from three weeks to four months. In the first group the symptoms are those of cirrhosis. Loss of appetite, loss of weight and strength, digestive disturbances, pallor and enlargement of the abdomen extend over a considerable period of time. Pain in the right side of the upper part of the abdomen is frequently complained of. At some stage a sudden exacerbation of symptoms is noted and the patient rapidly fails. Nausea and vomiting and hematemesis with slight jaundice become more marked, and on examination moderate enlargement of the liver with ascites and edema are found. Although it is said that the liver is enlarged in only 50 per cent of cases, our experience is that 75 per cent would be more accurate. As an important diagnostic point Brulé insists on fixity of the liver, so that it moves neither with palpation nor with respiration. In the second group the symptoms follow fairly closely the terminal stage of the previous group. The onset may be insidious, but there is a steady, rapid downward course with loss of weight and strength combined with progressive enlargement and fixity of the liver.

Jaundice was present in 65 per cent of Eggel's cases; it was only slight or moderate in all of our cases. There was marked ascites in three cases; in two there was practically none. Edema is rarely extreme and often absent. The spleen was enlarged in three cases, the enlargement in one being responsible for a diagnosis of Banti's disease. Fever is uncommon but may occur.[32] A summary of the clinical findings is shown in Table 1.

While the gross appearance of the liver is fairly typical there are several conditions with which it may be confused. It is of course

30. Rolleston, H. D.: Diseases of the Liver, Gallbladder and Bile Ducts, Ed. 2, London, Macmillan Company, 1914, p. 469.

31. Ewing, James: Neoplastic Diseases, Ed. 2, Philadelphia, W. B. Saunders Company, 1922, p. 682.

32. Ascoli, V.: Cancro primitivo del fegato a decorso febbrile, Sunto di due lezioni, Policlinico (sez. prat.) **31**:445-450 (April 7) 1924.

absolutely necessary to make a careful search for a primary focus, particularly in the suprarenals, breast, kidneys and gastro-intestinal tract. We mention the suprarenals first because, although true hypernephroma is a rare condition, its secondary manifestation in the liver may be identical with a primary carcinoma. We had one such case in which a huge nodular tumor in the liver with no apparent primary lesion elsewhere was responsible for a diagnosis of primary carcinoma. The absence of cirrhosis and the recognition microscopically of a clear celled structure in the looser parts of the tumor caused us to examine the suprarenals carefully. A small nodule, about 1.5 cm. in diameter, was found, and this proved to be an undoubted hypernephroma. Carcinoma of the kidney, or the condition commonly called hypernephroma, will also cause confusion. Tumor thrombi, necrosis, hemorrhages and various degenerations are common, while variations in color and general appearance, such as are found in cases of primary carcinoma, are deceptive. In general, secondary metastatic nodules occur nearer the surface; they are usually a homogeneous yellowish white when small, and tend to show hemorrhage and necrosis when large. The development of fibrous tissue within the nodules causes umbilication, such as is rarely seen in the primary form. There is rarely any accompanying cirrhosis, although slight local mild fibrosis may occur. In its absence the diagnosis of primary carcinoma should be made with the greatest hesitation. There are two other conditions to be guarded against, nodular hyperplasia and syphilis. Nodular hyperplasia is an uncommon condition in which the liver shows multiple adenomas throughout. While it is practically always associated with portal cirrhosis, cases have occurred in which it was absent. The same controversy has raged over the occurrence of cirrhosis with adenoma as with carcinoma, and the same writers express similar views concerning the two. Sabourin held that nodular hyperplasia was the intermediate stage between cirrhosis and carcinoma and that no distinction could be drawn between them. Muir [33] is in agreement with this. We have studied a case in which there was active hyperplasia in the liver cells, but in which one could say fairly definitely that there was no malignancy. The adenomas were an accentuation of the ordinary regenerating nodules and showed well marked hyperplasia, particularly at the periphery. With the tumor thrombosis which frequently occurs, the whole picture of a generalized cirrhosis with exaggerated "hobnails," some undergoing necrosis and softening, may closely resemble carcinoma. A microscopic examination is usually necessary to make the distinction, but even then it is often difficult. With

33. Muir, R.: On Proliferation of the Cells of the Liver, J. Path. & Bacteriol. **12**:287-305, 1908.

regard to syphilis little need be said. The adult form of hepar lobatum rarely causes trouble, but in children it must always be excluded.

We have divided our cases, according to the classification of Yamagiwa, into hepatoma arising from the liver cells and cholangioma arising from the bile ducts. With the possible exception of the massive form which rarely, if ever, arises from bile ducts, there is little to connect the gross and microscopic appearances, and the nodular and diffuse types may arise from both sources. There were four hepatomas and one cholangioma. Of the former, two were massive (Fig. 1), one was nodular (Fig. 2), and one diffuse (Fig. 3). The cholangioma was also

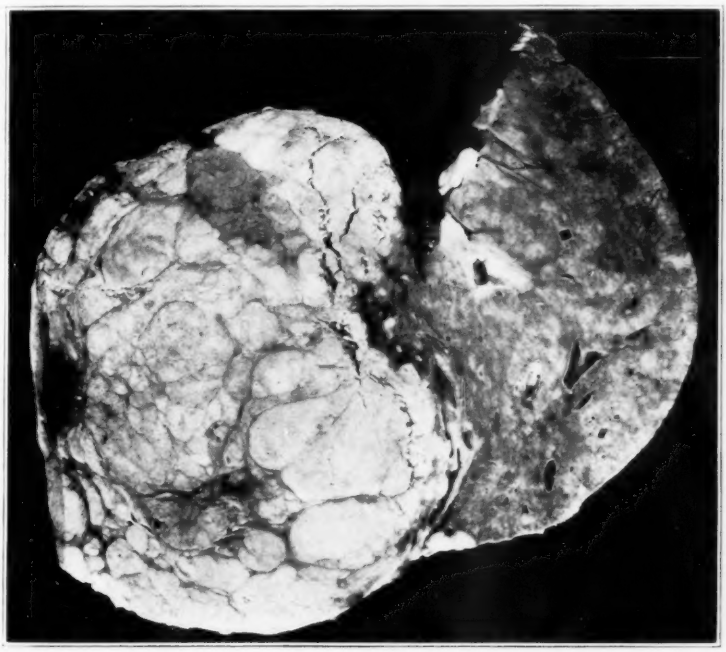

Fig. 1.—Massive hepatoma involving entire right lobe; fibrous tissue network in the tumor and little degeneration should be noted; cirrhosis in left lobe.

diffuse. While various criteria have been used to distinguish the two types, the difficulty of an accurate diagnosis has been emphasized by all writers. The presence of a well developed capillary stroma in the hepatoma form and its absence in the others constitute, according to von Heukelom, Wegelin [34] and Yamagiwa, the most reliable standard for

34. Wegelin, Karl: Ueber das Adenokarzinom und Adenom der Leber, Virchows Arch. f. path. Anat. **179**:95-153, 1905.

differentiation. Bile pigment within the cells in cases of carcinoma of the liver cells has been described by Ribbert.[35] The type of cell and its arrangement was considered important by Eggel. All of the hepatomas in the series presented cell types of great variety, from polygonal, polyhedral and triangular to round or oval, with cytoplasm staining rather more deeply and being more granular than is usual in normal liver cells.

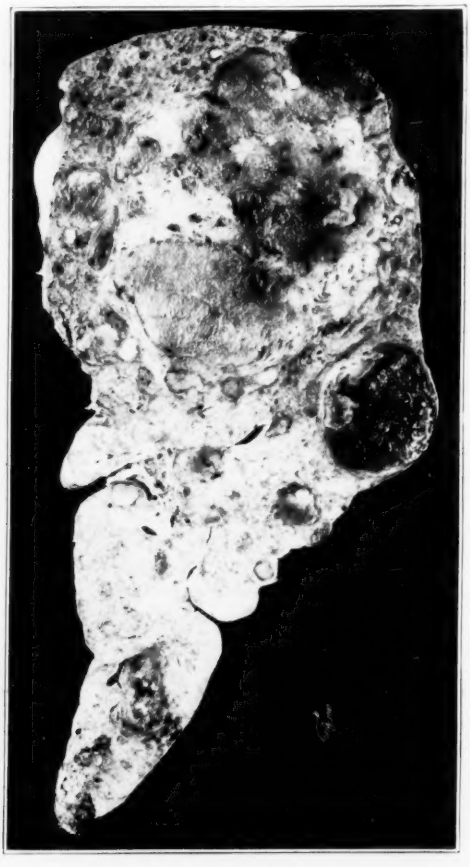

Fig. 2.—Nodular hepatoma showing nodules of various sizes in right lobe with hemorrhage and degeneration; tumor thrombosis and well marked cirrhosis in left lobe.

The nuclei varied greatly in size, some small and some large; all stained deeply; they often were irregular and pyknotic and exhibited frequent mitosis (Fig. 4). Giant cells often occurred containing from one to

35. Ribbert, Hugo: Das Maligne Adenom der Leber, Deutsche med. Wchnschr. **2**:1607-1609, 1909.

five nuclei. The arrangement in all four was trabecular, although in places an adenomatous formation was suggested. It is to be noted, however, that a lumen was not visible in any situation. Winternitz holds that this adenomatous formation is due to anaplasia from energy of cell growth. Rosettes, such as Goldzieher and von Bokay describe, were quite frequent. Bile pigment was present in a few cells, but not

Fig. 3.—Extensive diffuse primary hepatoma showing tumor thrombosis and well marked cirrhosis.

in metastatic areas, although it occurred in large amounts in the intercellular spaces and in the connective tissue at the periphery. A capillary stroma was certainly more prominent in this type of tumor, and it seemed as though the endothelium grew coincidently with it. In two

cases we noted large masses surrounded by endothelium (Fig. 5). Necrosis and dissociation of the cell columns was more common in the hepatoma, especially near the center of the nodules, while the structure was better preserved as the periphery was reached.

Concerning the cholangioma there are fairly well marked distinctions which enabled one to clinch the diagnosis. The cells were in general cuboidal or cylindrical, with clear or only finely granular protoplasm. They were only slightly larger than the cells of bile duct epithelium, but approximated fairly closely their general characteristics. The nuclei stained deeply and were hyperchromatic with a pyknotic tendency. Mitotic figures were much less common and the cells were better differentiated than in the liver cell type (Fig. 6). Their arrangement was

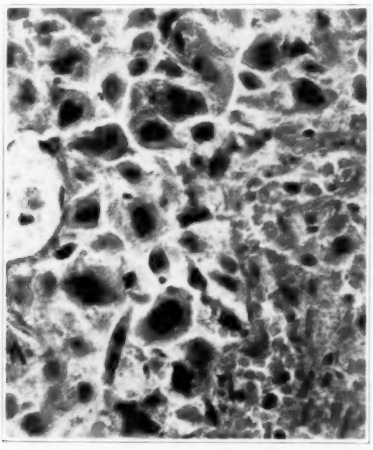

Fig. 4.—Hepatoma; malignant cells showing variation in size, mitotic figures, giant cells and marked capillary stroma, under higher power.

alveolar with definite lumina, often containing bile and biliary detritus. With regard to the stroma, capillaries were much less common, but a fine intercellular fibrous network was apparent (Fig. 7). In many situations this became concentrated to form dense bands or septums containing little in the way of either normal or malignant cells.

We would say that no one feature is sufficient to make a diagnosis of either type. Only after a careful examination of the most predominating characteristics can it be decided whether the resemblance is to the liver cell or to the bile duct cell. Cell type, stroma, general structure and behavior must all be carefully considered.

The question of the multicentric or unicentric origin of these tumors will not be discussed at length. In many areas direct invasion and

infiltration of the liver parenchyma by the malignant cells was observed (Fig. 8), and here the so-called transitional forms described by von Heukelom, Travis,[36] and others were seen. As we were able in every instance to decide which was a malignant cell and which a normal cell, and could find no true transitional forms we must agree with Winternitz, Karsner and others that the condition is unicentric in origin and that its spread in the liver is metastatic by way of the portal and the hepatic veins.

The occurrence of extrahepatic metastasis in two of the five cases bears out the figures of others as to its relative infrequency. In one case there was an extension through the hepatic veins into the inferior vena cava, right auricle and pulmonary artery, with small nodules in

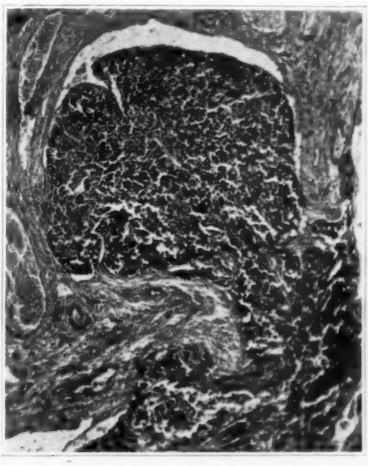

Fig. 5.—Hepatoma: solitary tumorous nodule in portal vein showing method of invasion; a layer of endothelium covers the tumor where it has retracted from vessel wall.

the lungs. It was similar to a case cited by Winternitz. In the second case there was extension to the spine at the level of the sixth dorsal vertebra. The body of this vertebra was replaced by a soft grayish pink mass, which had extended into the vertebral canal on either side of the cord, causing compression myelitis. This form of metastasis is unusual and we can find no parallel to it. In striking contrast to the comparative freedom from extrahepatic metastasis, the extension along the portal and the hepatic veins was remarkable.

36. Travis, Catherine H.: A Case of Multiple Primary Adenocarcinoma of the Liver with Cirrhosis, Bull. Johns Hopkins Hosp. **13**:108-111, 1902.

Relation to Cirrhosis.—Although the earlier statistics of Eggel, Goldzieher and von Bokay, and Yamagiwa showed the relation of cirrhosis to vary between 70 and 80 per cent, later work indicated that it is almost a constant feature and that it is the main predisposing factor in the condition. Particularly is this so in the hepatoma form in which, with few exceptions, cirrhosis is present. In the cholangioma form it is not so frequent, but large enough series have not been reported from which to judge. In our case the histories specifically point to cirrhosis in three, while the duration was short in two. It may well be that in those cases in which the onset was sudden the cirrhosis was of the silent type, giving no evidence of its presence until the onset of the malignancy. It is known that cirrhosis may exist for

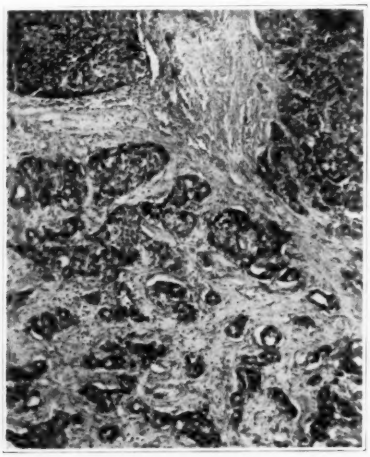

Fig. 6.—Cholangioma; cuboidal and cylindrical cells with tubulo-adenomatous structure buried in dense connective tissue stroma; resemblance to bile ducts and absence of capillary stroma should be noted; tumor thrombosis in left upper corner.

long periods and may be discovered only at postmortem examination. In Karsner's twelve cases it was present in all. In Winternitz' cases and in nineteen cases occurring since 1922, all showed cirrhosis. The case of Helvestine is the only one in which it was absent.

The cirrhosis itself has been attributed to many factors: alcohol, syphilis, cardiac disorders [13] and schistosomiasis.[37] With regard to the last an interesting fact has been brought to light; in black races, in which cirrhosis due to schistosomiasis is common, primary carcinoma

37. Pirie, J. H. H.: Hepatic Carcinoma in Natives of Africa and Its Frequent Association with Schistosomiasis, M. J. S. Africa **17**:87-97 (Dec.) 1921.

is also relatively common as compared with other forms of malignant disease. Carcinoma in native races is of course extremely rare. Thus, of twenty-one cases of carcinoma which von Hansemann [38] was able to record in the examination of 200,000 natives in the German colonies of Africa, four were primary in the liver, a figure out of all proportion to other forms. In the same way Pirie [37] found in South African natives thirty-six primary in the liver out of ninety-one of all forms, and of these ten were proved to have had previous cirrhosis due to schistosomiasis.

The points at issue are whether the cirrhosis is primary, secondary or coincident with the carcinoma. It seems that the last view is untenable from the regularity of the appearance of cirrhosis and from its generalized and often severe form. Hanot and Gilbert advanced this

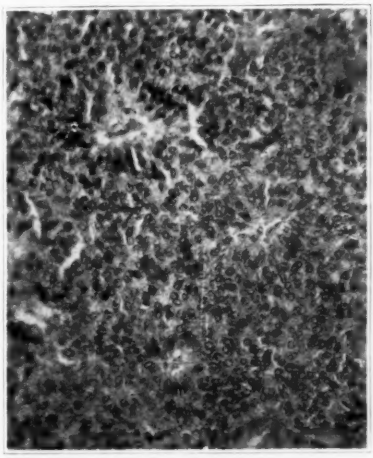

Fig. 7.—Cholangioma; more solid structure than in Figure 6 with frequent rosettes containing biliary detritus, under higher power; regularity of cells and absence of mitotic figures should be noted.

view on the ground that the tumor and the cirrhosis were produced by the same etiologic agent. From an analogy with other cases of carcinoma in which chronic inflammatory processes are almost certainly etiologic, such a view would be hard to maintain, particularly when we examine cases in which cirrhosis has undoubtedly preceded the malignant change. In support of the view of Lancereaux, Wegelin and others, that the presence of a malignant tumor induces cirrhosis, even less can be said. We know that metastatic growths or indeed any

38. Von Hansemann, D.: Ueber das Vorkommen von Geschwülsten in den Tropen, Ztschr. f. Krebsforsch. **14**:39-45, 1914.

form of growth will cause localized fibrosis or cirrhotic processes in the liver as in any other organ, but never the generalized and severe form so often seen in this condition. Winternitz has shown, and our cases bear out his statement, that the process is often more severe in proximity to the tumors, but such a thing is easy to understand on the basis that they act as a mild local irritant, just as any foreign body would do. We are in full accord with the view that the cirrhosis is the primary factor and that the malignancy is a change superimposed on it. This has been upheld by von Heukelom, Eggel, Goldzieher and von Bokay, Rolleston [39] and many others.

The powers of regeneration possessed by the liver are remarkable. Von Podwyssozki [39] first showed that removal of large pieces of liver was quickly followed by regeneration. Mann [40] has removed four-fifths

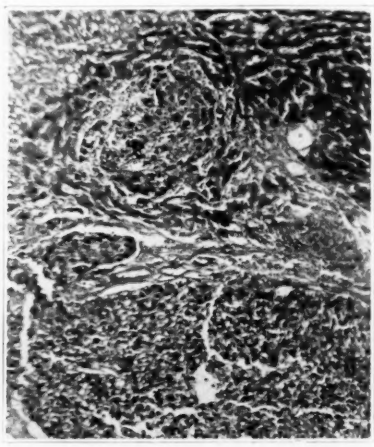

Fig. 8.—Hepatoma; tumor thrombus in portal vein; so-called transitional area adjacent to thrombus showing compressed and atrophic liver cells, dense connective tissue and lymphocytic invasion; the tumor cells can be distinguished in the sinusoids.

of a dog's liver with complete regeneration in six weeks. Such regeneration is by cell hyperplasia rather than by cell hypertrophy. In cirrhosis Kretz [41] was the first to point out that the process was one of destruction of the liver cells and that regeneration was a most marked feature. Thus in cirrhosis there is an active destruction and an equally active regeneration with an adenomatous condition of the liver cells

39. Von Podwyssozki, W.: Experimentelle Untersuchungen über die Regeneration der Drüsengewebe, Beitr. z. path. Anat. u. z. allg. Path. **1**:259-360, 1886.

40. Mann, F. C.: Studies in the Physiology of the Liver: I, Technic and General Effects of Removal, Am. J. M. Sc. **161**:37-42 (Jan.) 1921.

41. Kretz, R.: Cirrhosis of the Liver, Internat. Clin. **3**:289-297, 1905.

most pronounced in the portal type. Buried in the connective tissue are bile canaliculi about which so much controversy has arisen. Mac Callum [42] and others regard them as bile ducts and ascribe to them the power of producing liver cells. The latter is denied by Muir [33] and by Mallory.[43] Rolleston is of the opinion that they are not bile ducts, but atrophic liver cells. We agree with Muir in this connection that the bile canaliculi are of biliary origin, but that they do not form liver cells. From a close microscopic examination of many cirrhotic livers we have never convinced ourselves that regeneration of liver cells from bile ducts takes place. Whatever the factors may be producing the cirrhosis, it seems that the path of entry is either the portal or biliary channels. Undoubtedly the most common is the portal, and consequently the annular type of cirrhosis is more frequent. In a mild case destruction

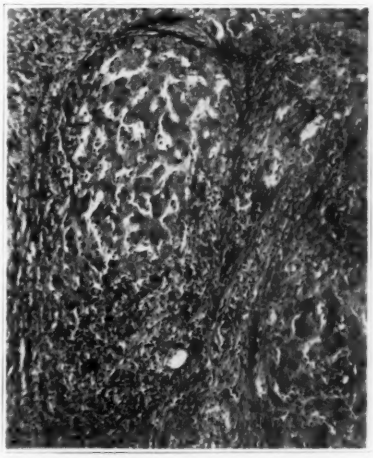

Fig. 9.—Cirrhosis, showing active destruction of peripheral cells with formation of hyperplastic adenomatous nodules; absence of central veins and radiate arrangement, and extensive lymphocytic invasion in connective tissue stroma adjacent to hepatic cells should be noted.

of cells takes place in the neighborhood of the portal spaces with the consequent laying down of new connective tissue. For the continuation of this process two factors are necessary, cell death with the continuous action of a toxin. The rapidity of the process and its extent will be profoundly modified by a third factor, regeneration of the liver cells and bile ducts.

42. MacCallum, W. G.: Regenerative Changes in Cirrhosis of the Liver, J. A. M. A. **43**:649-654 (Sept. 3) 1904.

43. Mallory, F. B.: Cirrhosis of the Liver; Five Different Types of Lesions from Which It May Arise, Bull. Johns Hopkins Hosp. **22**:69-75, 1911.

The gross appearance of the liver will vary with the balance maintained by these three factors. We believe that in the portal type the toxic agent affects primarily the cells in the neighborhood of the portal spaces and, second, any group of cells without reference to the so-called hepatic lobule. In the biliary type the toxic agent is more confined to the bases of Sabourin's acini; this results in a more anatomic distribution and so-called monolobular fibrosis. Nevertheless, we would emphasize the fact that in neither type is the classification of cirrhosis based on an anatomic appearance justifiable, for the process is governed by the resultant of the three factors mentioned, and not by the influence of the individual lobule. As biliary cirrhosis is more uncommon and less severe than portal cirrhosis regeneration is not such a marked feature, and hence it is rarely seen in conjunction with carcinoma. In the portal type, for instance, a small section of a lobule, or even large masses of liver cells, are destroyed, often including several hepatic lobules, but, provided a few healthy cells remain, the most extraordinary regeneration will take place.

We have indicated in the microscopic examination of these cases the large nodules of liver tissue devoid of central veins and any radiate arrangement, which could only have been produced by a complete new formation. Evidence of the regeneration is found at the periphery of the nodule. The cells are larger, more convex, with clearer and more acidophilic cytoplasm. The nuclei are larger, often double, and occasional mitotic figures can be detected (Fig. 9). MacCallum [44] has demonstrated this last point clearly. In short, we have primarily an active cell destruction with coincident cell regeneration and formation of hyperplastic adenomatous areas of liver tissue. Within the connective tissue the same hyperplastic change is proceeding, with a tremendous increase in the number of bile canaliculi. The same evidence of hyperplasia can be seen in them. Whether the budlike structures sometimes seen at their ends are liver cells we are not prepared to say. If it were so and we ascribe to both the liver cells and the bile ducts the power of malignant proliferation, then we must admit the possibility of liver cell carcinoma arising from bile ducts. There is no doubt that embryologically liver cells and bile ducts have a common origin, but we believe that in the fully differentiated organism, cells which were originally totipotent become and remain unipotent. In short, to take the case in point, bile ducts produce bile ducts and carcinoma of bile ducts, while liver cells are capable of reproducing themselves to the point of malignancy. The first step in the chain of events is the long continued chronic hepatitis with adenoma formation. Most cases are of this type.

44. MacCallum, W. G.: Regenerative Changes in the Liver After Acute Yellow Atrophy, Rep. Johns Hopkins Hosp. **10**:375-384, 1902.

If the process is extremely rapid or if the regenerative capacity of the cells is great, nodular hyperplasia occurs. As has been pointed out, many writers make no distinction between this and carcinoma, holding that the three conditions, adenoma, nodular hyperplasia and carcinoma, insensibly shade off into each other. Such an occurrence would be easy to understand were it not for the great rarity of carcinoma compared with the frequency of cirrhosis and regenerative changes in the liver. There can be little doubt that some specific factor or specific change in environment is responsible for the transition from hyperplastic to malignant cells, a condition that removes the barrier confining their biologic function of growth and regeneration within normal limits. Be that as it may, we are of the opinion that from 3 to 4 per cent of cases of cirrhosis will develop into carcinoma as the direct result of the changes induced by the chronic inflammatory condition.

SUMMARY AND CONCLUSIONS

1. Five cases of primary carcinoma of the liver are described: four hepatomas or primary carcinomas of the liver cells and one cholangioma or carcinoma of the bile ducts.

2. The gross and microscopic appearances have little in common, so the massive, nodular and diffuse forms appear in any microscopic type. The massive form is, however, usually derived from liver cells.

3. All the cases were associated with intrahepatic metastasis by way of the portal and the hepatic veins, but in only two were there extrahepatic growths.

4. No transitional forms were found and the origin is therefore believed to be unicentric.

5. All the cases were associated with cirrhosis of the portal type. The histories in three cases point to a preceding cirrhosis, and in all the microscopic examination of the connective tissue indicated a long standing process.

6. The relation between the hyperplastic liver tissue and the malignant cells is emphasized.

7. From 3 to 4 per cent of cases of cirrhosis, particularly of the portal type, will become carcinomatous.

THE EFFECT OF LIVER DAMAGE ON CHOLECYSTOGRAPHY IN DOGS BY THE USE OF SODIUM TETRA-IODOPHENOLPHTHALEIN *

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AND
L. R. WHITAKER, M.D.
BOSTON

The visualization of the gallbladder for diagnostic purposes was made possible through the observation of Abel and Rowntree [1] that halogen compounds of phenolphthalein are excreted almost wholly by the liver. Application of this principle to cholecystography was first made by Graham and Cole,[2] who found that by injecting intravenously tetra-iodophenolphthalein and tetrabromphenolphthalein the gallbladder could be rendered opaque to roentgen rays and therefore visible.

Tetra-iodophenolphthalein was first condemned by Graham and Cole as being too toxic for clinical use; probably they employed an impure product. Observations from this laboratory, however, have shown that with a more purified salt the toxicity of both compounds is the same. But since the opacity to roentgen rays of sodium tetra-iodophenolphthalein is twice as great as that of tetra-bromphenolphthalein a good shadow of the gallbladder can be obtained by a dose only one-half as large. This means that the iodin salt is practically only one-half as toxic, and therefore to be preferred for cholecystography.

Theoretically the visualization of the gallbladder by these drugs is based on the power of this organ to concentrate substances excreted by the liver in the bile.

For the purpose of the test a sterile solution in distilled water of sodium tetra-iodophenolphthalein is injected intravenously and roentgenograms of the gallbladder taken from about eight to ten hours following the injection, at which time the concentration of the drug in the gallbladder is supposed to be at its height. A fairly good shadow of the gallbladder is also obtained by the oral administration of the drug in "enteric" coated pills, devised in the clinics of the Peter Bent Brigham Hospital. Details concerning the subject have been published

* From the Laboratory for Surgical Research, Harvard Medical School.

1. Abel and Rowntree: J. Pharmacol. & Exper. Therap. **1**:233, 1909.

2. Graham, E. A., and Cole, W. H.: Roentgenologic Examination of the Gallbladder, J. A. M. A. **82**:613 (Feb. 23) 1924. Graham, E. A.; Cole, W. H., and Copher, G. H.: Visualization of the Gallbladder by the Sodium Salt of Tetrabromphenolphthalein, J. A. M. A. **82**:1777 (May 31) 1924.

elsewhere.[3] Suffice it to say here that one or the other of these methods have been utilized in about 200 cases at the Peter Bent Brigham Hospital with very good results. With twenty-eight patients upon whom operations were performed, this method of diagnosis proved to be correct in 93 per cent. Other clinics, including that of Dr. E. A. Graham, have made similar reports.

The purpose of this study was twofold: (1) to determine the effect of sodium tetra-iodophenolphthalein on animals in the presence of an experimentally damaged liver (since as noted in the foregoing the salt is normally excreted almost wholly by the liver), and (2) on the production of cholecystograms by use of this drug under similar conditions.

MATERIAL AND METHODS

It has been shown by numerous workers in this country as well as in Europe that when administered to various animals—cats, dogs, rabbits—chloroform acts as a poison leading to degenerative changes of the viscera, particularly the liver. The lesions found in the liver are usually defined as central necrosis or fatty degeneration. Of special interest in that respect are recent experiments by Davis and Whipple[4] to the effect that the degree of liver damage depends: (1) on the diet on which the animal was kept prior to the experiment, and (2) on the amount of chloroform used during anesthesia. In the experience of these workers chloroform given after fasting will produce hepatic damage that is in direct proportion to these two factors. "The uniformity of liver injury produced in a dog after three to four days of fasting," say these authors, "is remarkable." In their experiments they used mostly puppies from 5 to 6 pounds (about 2 kg.) in weight. The animals were starved for from three to four days and then kept under anesthesia for from sixty to seventy-five minutes. The amount of chloroform given was not stated in their protocols.

In the experiments that follow, the methods of Whipple and Davis for the production of damage of the liver by chloroform were utilized.

Dogs were used in all the experiments. The animals were isolated and kept on water only for from one to three days. At the end of the starving period they were anesthetized with chloroform by the drop method. The duration of the anesthesia as well as the amount of chloroform used is indicated in the tables. Twenty-four hours after the administration of chloroform, when the liver damage usually reaches

3. Whitaker, L. R., and Milliken, G.: A Comparison of Sodium Tetrabromphenolphthalein with Sodium Tetra-Iodophenolphthalein in Gallbladder Radiography, Surg., Gynec. & Obst. **40**:17 (Jan.) 1925. Milliken, G., and Whitaker, L. R.: The Clinical Use of Sodium Tetra-Iodophenolphthalein in Cholecystography, Surg., Gynec. & Obst. **40**:646 (May) 1925.

4. Davis, N. C., and Whipple, G. H.: The Influence of Fasting and Various Diets on the Liver Injury Effected by Chloroform Anesthesia, Arch. Int. Med. **23**:613 (May) 1919.

its maximum, a sterile 10 per cent. solution of sodium tetra-iodophenolphthalein in the amount of 0.18 gm. per kilogram of body weight [5] was injected intravenously. Cholecystography was performed from six to eight hours after the injection of tetra-iodophenolphthalein, at which time the concentration of the drug approaches its maximum, and therefore renders the gallbladder opaque. Most of the animals were operated on for the purpose of obtaining a piece of liver for histologic examination, usually about thirty-six hours after the administration of chloroform and from twelve to sixteen hours after the injection of tetra-iodophenolphthalein. Tissues were fixed in formaldehyd, stained with the usual stains, and with Herxheimer's stain for the presence of fat.

TABLE 1.—*Summary of First Series of Experiments*

Dog*	Weight, Kg.	Fasting Period, Days	Amount of Chloroform Used, C.c.	Duration of Narcosis, Minutes	Amount of Drug Used per Kilogram of Animal Weight, Gm.	Cholecystography	Pathologic Changes in Liver
1	6.0	3	40	60	0.18	Negative	Extensive central necrosis and fatty degeneration (Fig. 1)
2	7.0	3	60	60	0.18	Negative	Extensive central necrosis; slight fatty degeneration
3	7.5	3	45	60	0.18	Negative	Same as Dog 2 (Fig. 2)
4	7.1	3	25	60	0.18	Negative	About one-half of liver lobule necrotic; fatty infiltration of remaining cells
5	5.4	3	18	30	0.18	Negative	About one-third of liver lobule necrotic; fatty infiltration
6	6.1	3	12	30	0.18	Negative	Same as Dog 5 (Fig. 3)

* All dogs used were puppies.

EXPERIMENTS

The experiments can be divided into three series according to the weight of the animals, the amount of chloroform used in each instance, the duration of narcosis, and the starving period.

Series 1 includes six young dogs (Dogs 1-6).

From Table 1 it will be seen that (1) animals used in these experiments were from 5.4 to 7.1 kg. in weight; (2) the duration of narcosis was from one-half hour (Dogs 5 and 6) to one hour; (3) the amount of chloroform given varied, in Dog 5 being only 18 c.c., and in Dog 6 only 12 c.c.[6] It is also to be noted (4) that none of the animals of this

5. This is the optimum dose for producing an opaque gallbladder in healthy dogs, in which this amount is well tolerated. Furthermore, it has been found in this laboratory that 0.18 gm. of the drug per kilogram of body weight produced no hepatic lesions unless repeated at intervals of a few days over a period of several weeks.

6. In previous experiments of Whipple and Sperry (Whipple, G. H., and Sperry, J. A.: Chloroform Poisoning, Liver Necrosis and Repair, Bull. Johns Hopkins Hosp. **20**:278 [Sept.] 1909) about 45 c.c. of chloroform were always used.

series gave a positive shadow of the gallbladder, and (5) that the dogs with a damaged liver usually tolerated the drug well.

Figures 1, 2 and 3 show photomicrographs from liver sections taken from Dogs 1, 3 and 6. As seen from the figures, in Dog 1 only a narrow margin of normal appearing liver remained around the portal areas. The rest of the liver lobule was necrotic. In Figure 2 the liver lobule can be divided into three zones: (1) periportal, consisting of normal appearing liver cells which are, however, infiltrated with fat; (2) a narrow band of cells showing complete fatty degeneration, and (3) central necrosis, which occupies more than one-half the lobule.

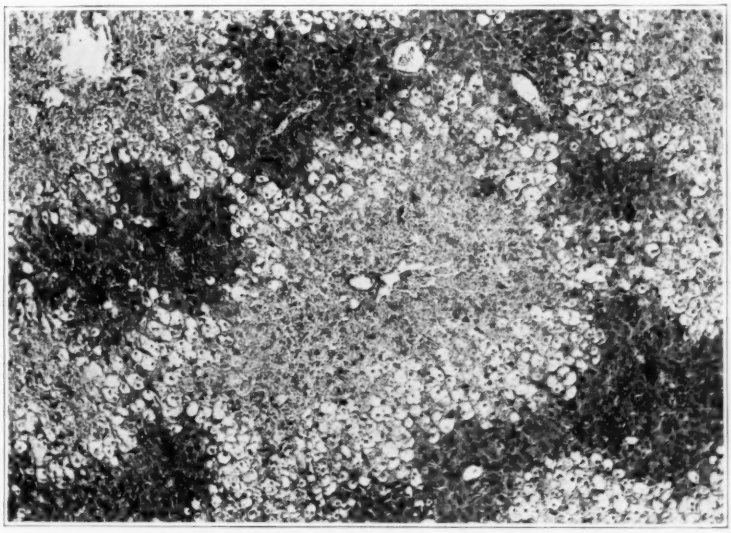

Fig 1 (Dog 1).—Section of liver showing extensive central necrosis and fatty degeneration; hematoxylin-eosin; × 80.

In Figure 3 almost one-half the lobule shows fatty infiltration. There are a few necrotic cells around the central vein.

From the first series of experiments we therefore conclude:

1. Cholecystography with sodium tetra-iodophenolphthalein in dogs in which the liver lobule is extensively damaged by chloroform (fatty degeneration, central necrosis) is negative.

2. Dogs in which the liver shows a central necrosis occupying more than one-half the lobule as a rule tolerate the drug fairly well.[7]

7. It is interesting to note here that in complete biliary obstruction the toxicity of the drug, as shown in this laboratory by Maddock and Whitaker, is only slightly increased.

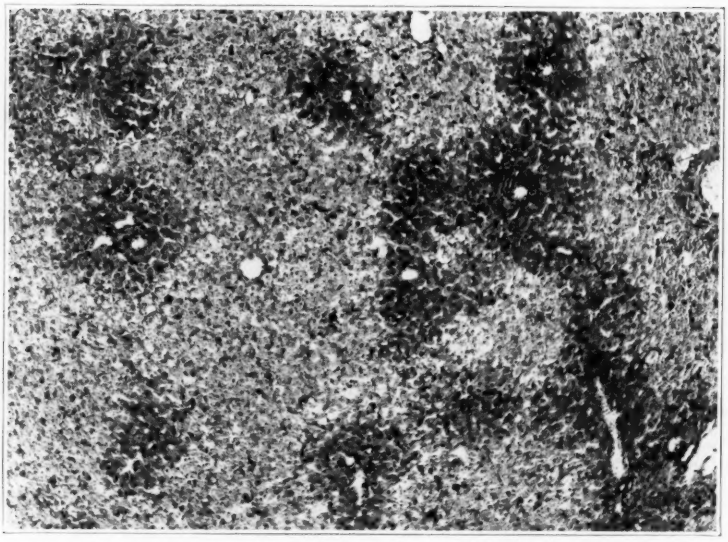

Fig. 2 (Dog 3).—Section of liver showing central necrosis of about one-third the liver lobule; hematoxylin-eosin; × 80.

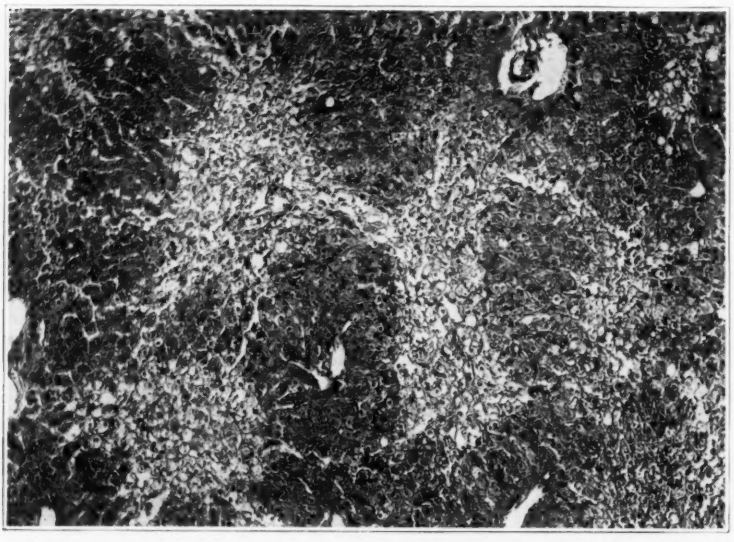

Fig. 3 (Dog 6).—Section of liver showing fatty degeneration of more than one-half the liver lobule; hematoxylin-eosin; × 80.

3. Starvation for three days followed by the administration of small amounts of chloroform (12 c.c. in one case) over one-half hour leads to an extensive damage of the liver.

SECOND SERIES OF EXPERIMENTS

The second series of experiments differed from the first in that (1) full grown large dogs were used; (2) the fasting period was reduced from three days to twenty-four hours, and (3) the amount of chloroform administered was in some instances reduced to a minimum of 6 c.c.

The purpose of these experiments was to produce a *minimum* liver damage and thus to ascertain whether such a liver would excrete the drug in an amount sufficient to render the gallbladder visible.

TABLE 2.—*Summary of Second Series of Experiments*

Dog	Weight, Kg.	Fasting Period, Days	Amount of Chloroform Used, C.c.	Duration of Narcosis, Minutes	Cholecystography	Pathologic Changes in Liver
7	7.4	1	20	30	Negative	Extensive central necrosis; fatty infiltration of remaining cells (Fig. 4)
8	9.0	1	10	20	Positive	Central necrosis, about one-sixth of liver lobule
9	13	1	11	20	Positive	A few necrotic cells around the central vein; fatty infiltration, one-third of lobule
10	17	1	10	10	Positive	Fatty infiltration, about one-fourth of liver lobule
11	14.8	1	10	10	Positive (faint)	Fatty infiltration; no necrosis (Fig. 5)
12	13.8	1	12	10	Positive	Fatty infiltration, about one-half of lobule (Fig. 6)
13	17	1	12	12	Positive (faint)	Almost similar to Dog 12 (Fig. 7)
14	12.3	1	6	10	Positive	Fatty infiltration, about one-third of liver lobule
15	20	1	6	10	Positive	Almost similar to Dog 14

Of the series of nine dogs only one (Dog 7) gave a negative cholecystogram. Figure 4 shows the large amount of liver damage produced in this animal. It is of interest to note that only 20 c.c. of chloroform administered during a period of thirty minutes produced an extensive lesion of the liver in an animal that had been fasting for twenty-four hours. Of the other eight animals two gave faint shadows, and six were frankly positive. In two of those with faint shadows the liver, as illustrated in Figure 6, showed considerable damage.

A fatty infiltration of the liver mostly confined to the central area but also conspicuous all over the lobule was likewise observed in Dogs 8, 13, 14 and 15, all of which gave frankly positive cholecystograms, showing that a shadow can be obtained in the presence of fairly extensive damage to the liver.

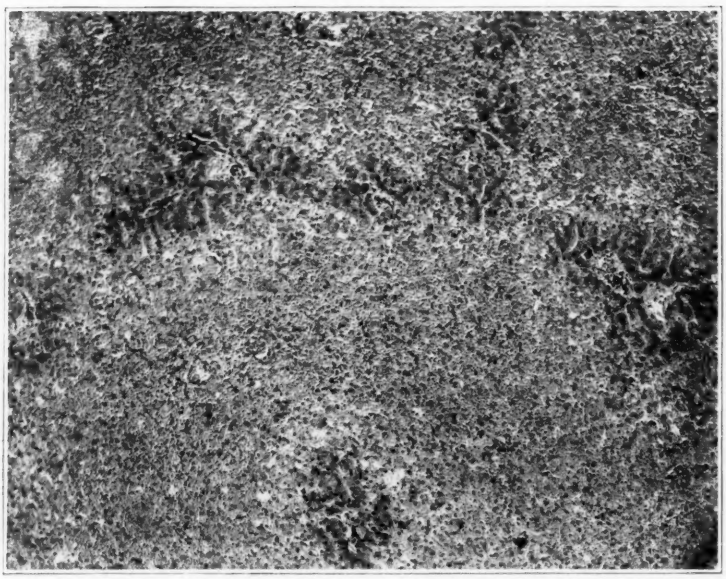

Fig. 4 (Dog 7).—Section of liver showing extensive central necrosis and fatty infiltration of remaining cells; hematoxylin-eosin; × 80.

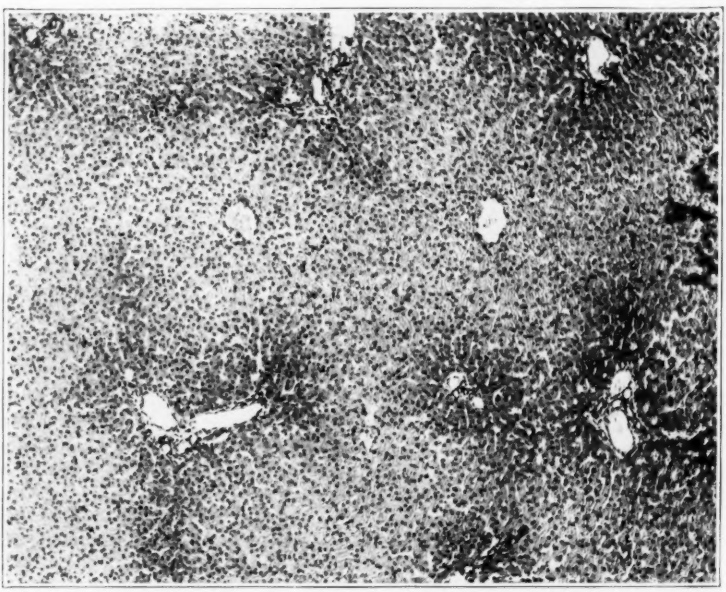

Fig. 5 (Dog 11).—Section of liver showing fatty degeneration of more than one-half the liver lobule; hematoxylin-eosin; × 80.

Figure 5 (Dog 11) shows the amount and type of injury to the liver produced by only 10 c.c. of chloroform. Figure 6 illustrates the extent of degeneration compatible with a positive cholecystogram.

These experiments confirm in general those made by Davis and Whipple. It may be added that in starved animals quite extensive necrosis was produced with much smaller doses of chloroform than used by these workers.

From this second series of experiments we conclude, as in the first series, that an extensive lesion of the liver interferes with its excretory

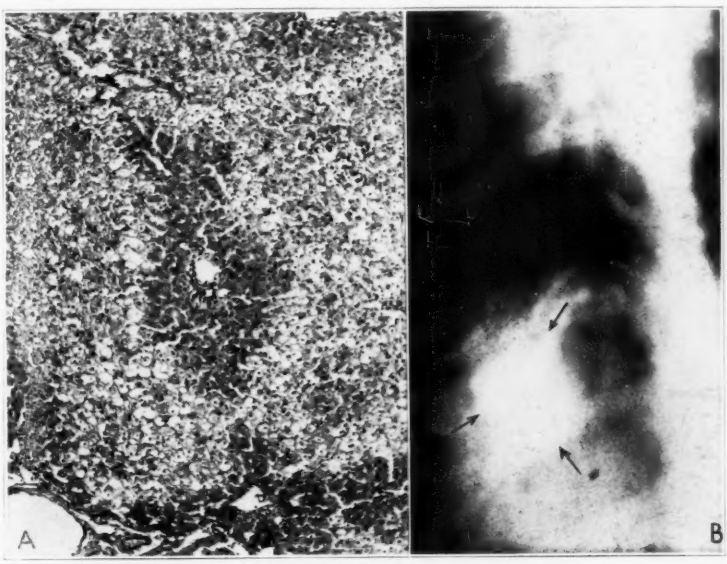

Fig. 6 (Dog 12).—*A*, section of liver showing fatty degeneration of about one-half the liver lobule; hematoxylin-eosin; × 80; *B*, cholecystogram showing positive shadow of gallbladder (arrows) in same dog.

function, and consequently with cholecystography, but that a moderately damaged liver (slight central necrosis; fatty infiltration) does not interfere with cholecystography in dogs by the use of sodium tetraiodophenolphthalein (Fig. 6).

THIRD SERIES

The third series includes six dogs (Dogs 16-21) that were not subjected to starvation, having been anesthetized with chloroform immediately after taking food.

Table 3 shows the amounts of chloroform given and the duration of the narcosis. It will be seen that the amount of chloroform administered

TABLE 3.—*Summary of Third Series of Experiments*

Dog	Weight, Kg.	Fasting Period	Amount of Chloroform Used, C.c.	Duration of Narcosis, Minutes	Cholecystography
16	10	None	10	10	Positive
17	15	None	15	10	Positive
18	19	None	15	15	Positive
19	20	None	15	15	Positive
20	19.3	None	18	17	Positive
21	14.2	None	15	15	Positive

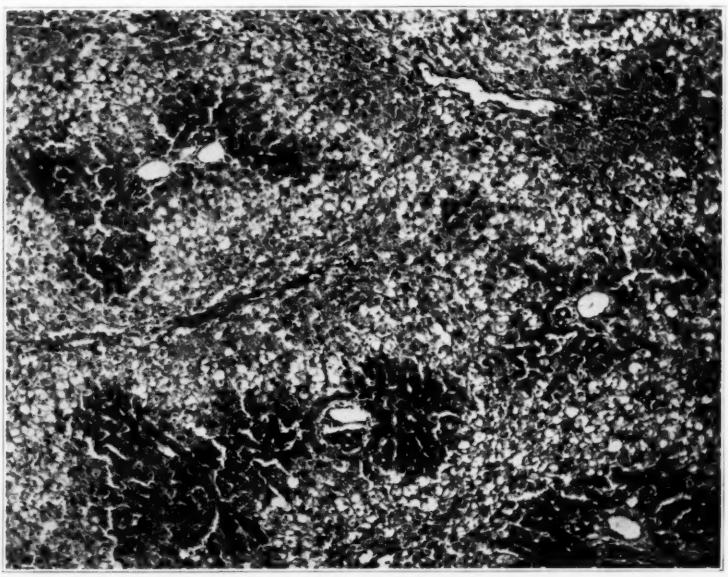

Fig. 7 (Dog 13).—Section of liver showing changes almost identical with those in Dog 12 (Fig. 6).

to each dog of this series is higher than that used in the second series of experiments, with the exception of Dog 7. The duration of narcosis was from ten to seventeen minutes. On histologic examination the livers of three animals that were killed (Dogs 16, 20 and 21) showed a narrow central zone of fatty infiltration with an occasional polymorphonuclear leukocyte. In all the six animals clear shadows of the gallbladder were obtained. There is, then, an additional interest in this series of experiments, since it shows that starvation is apparently one of the primordial factors in the production of liver damage by the use of chloroform.

CONCLUSIONS

The purpose of the present study has been (1) to determine the toxicity of sodium tetra-iodophenolphthalein in dogs in which the liver was damaged by the use of chloroform as an anesthetic, and (2) the degree of excretion of the drug by a liver thus damaged as determined by cholecystography.

The results have shown that a moderately damaged liver does not interdict the use of sodium tetra-iodophenolphthalein, for the animals with a liver so damaged tolerate the drug apparently as well as normal animals.

Because of the much smaller amount of sodium tetra-iodophenolphthalein routinely used in patients (one-fourth) than used with safety on dogs known to have more or less extensive hepatic damage, the existence of presumed or obvious hepatic disease in human beings should not necessarily preclude the employment of the drug for purposes of cholecystography in the clinic.

CARBON MONOXID POISONING

A COMPARISON OF THE PRESENT METHODS OF TREATMENT*

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In 1920 Henderson and Haggard [1] published the results of experimental treatments of dogs asphyxiated by carbon monoxid. The rate of fall of the carbon monoxid hemoglobin was studied in control dogs and in dogs treated with air containing 6 per cent. and 10 per cent. carbon dioxid and with oxygen containing 10 per cent. carbon dioxid. As a result of these experiments they advocated the use of a mixture of carbon dioxid in oxygen for carbon monoxid poisoning. In later papers [2] the same workers have recommended the use of a mixture of 95 per cent. oxygen and 5 per cent. carbon dioxid for resuscitation from carbon monoxid asphyxia, from ether or alcohol intoxication and for respiratory failure due to other causes, such as morphin narcosis. In the discussion following the reading of one of these papers,[3] Dr. R. R. Sayers, chief surgeon of the Bureau of Mines, stated that at that stage of his experiments pure oxygen seemed to give better results as to relief of symptoms, and almost as rapid elimination of carbon monoxid from the blood as did the carbon dioxid-oxygen mixture.

Sayers and Yant [4] as a result of experiments on human subjects now recommend the use of pure oxygen, and also recommend that physicians use the carbon dioxid-oxygen mixture when possible, and note the results. It is to be remarked here that the carbon dioxid-oxygen mixture they used contained approximately 10 per cent. of carbon dioxid.

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* From the Medical Research Division of Edgewood Arsenal.

1. Henderson, Yandell; and Haggard, H. W.: The Elimination of Carbon Monoxid from the Blood After a Dangerous Degree of Asphyxiation, and a Therapy for Accelerating the Elimination, J. Pharm. & Exper. Therap. **16**:11-20 (Aug.) 1920.

2. Henderson, Y.; Haggard, H. W., and Coburn, R. C.: The Therapeutic Use of Carbon Dioxid After Anesthesia and Operation, J. A. M. A. **74**:783-786 (March 20) 1920. Henderson, Y.: Resuscitation from Carbon Monoxid Asphyxia, J.A.M.A. **83**:758-763 (Sept. 6) 1924. Haggard, H. W., and Henderson, Y.: The Treatment of Carbon Monoxid Poisoning, J. A. M A. **77**:1065-1067 (Oct. 1) 1921.

3. Haggard and Henderson (Footnote 2, third reference).

4. Sayers, R. R., and Yant, W. P.: The Elimination of Carbon Monoxid from Blood, by Treatment with Air, with Oxygen, and with a Mixture of Carbon Dioxid and Oxygen, Pub. Health Rep. **38**:2053-2074 (Sept. 7) 1923.

More recently, Haldane,[5] while stating that the carbon dioxid-oxygen mixture is the best known remedy for carbon monoxid poisoning points out that the great weight of the gas cylinders required is a disadvantage. In the absence of the carbon dioxid-oxygen cylinders he recommends the use of a carbon dioxid-air mixture, and describes an easily portable apparatus that will give a from 3 to 5 per cent. mixture of carbon dioxid in air for five hours. He also states that this treatment gives immediate relief from severe symptoms and probably washes out carbon monoxid from the body more rapidly than if pure oxygen were used. Henderson, Haggard and Coburn[6] also have described a simple apparatus for administering carbon dioxid-air mixtures.

Nicloux, Nerson, Stahl and Weill[7] disagree with the findings of Henderson and Haggard so far as the superiority of air plus carbon dioxid, 5 per cent. over pure oxygen, is concerned. They found oxygen to be superior to the carbon dioxid-air mixture. They also found that oxygen plus 5 per cent. of carbon dioxid acts slightly faster than pure oxygen in washing out carbon monoxid from the blood. It has been seen that Henderson and Haggard worked with a 10 per cent. mixture of carbon dioxid in oxygen, while Nicloux and his co-workers used a 5 per cent. mixture of carbon dioxid in oxygen or air. It is therefore impossible to compare the curves of Nicloux and Henderson as they were using different percentages of carbon dioxid, but it is of interest to note that Nicloux failed to obtain marked results when using 5 per cent. carbon dioxid mixtures in oxygen or air, which are the mixtures suggested for clinical use by Henderson and Haldane, respectively.

Henderson[8] states that dogs are less sensitive to stimulation of breathing by carbon dioxid than are men, and that while 5 per cent. carbon dioxid does not cause an active breathing in normal men it is sufficient to stimulate the respiratory center of persons who have been asphyxiated.

From the foregoing review it may be seen that there is a difference of opinion as to the relative value of the different treatments. An exact determination of their value would require a number of highly dangerous experiments on men, in which the percentage of hemoglobin saturated with carbon monoxid would rise as high as 65 or 70 per cent., and this is impracticable. The necessity for an exact evaluation arises

5. Haldane, J. S.: The Use of Carbon Dioxid as a Remedy in Gas Poisoning, Colliery Guardian **128**:1633-1634 (Dec. 24) 1924.

6. Henderson, Haggard and Coburn (Footnote 2, first reference).

7. Nicloux, M.; Nerson, H.; Stahl, J., and Weill, J.: Sur l'élimination de l'oxyde de carbone aprés intoxication grave: influence de la respiration de l'air on de l'oxygéne additionnee de 5 P. 100 d'acide carbonique, Compt. rend. Soc. de biol. **92**:178-182 (Jan. 30) 1925; Sur l'élimination de l'oxyde carbone aprés intoxication grave, ibid. **92**:171-178 (Jan. 30) 1925.

8. Henderson, Yandell: Personal communication to authors.

from the desirability of deciding on the type of apparatus to be kept at hand. In military practice carbon monoxid asphyxia cases occur most often in military land mines or after powder explosions on warships. In these locations pure oxygen is usually available; in the land mines it can be obtained from the oxygen rescue apparatus kept at the pit-heads, and aboard ship from the oxy-welding outfits. Carbon dioxid is not available at these places and special supplies would have to be provided as in the "sparklet" method described by Briggs [9] or a similar apparatus made by the Siebe Gorman Company.[10] If such apparatus is at hand the oxygen cylinders can be enriched with the desired percentage of carbon dioxid. Both oxygen and carbon dioxid can be easily procured in cities, and as Henderson has pointed out, any intern can quickly rig up an apparatus that will give the required mixture. In any event the weight of cylinders and apparatus required is great, and if the portable air plus carbon dioxid apparatus designed by Haldane is effective its use would be indicated in localities where transportation is difficult.

In an effort to determine the relative value of the suggested treatments the following experiments have been performed.

EXPERIMENTAL PROCEDURE

Dogs were used for the experiments. Under local anesthesia (cocain) the femoral veins were exposed, the legs bandaged and the animals were then gassed in a continuous flow gassing chamber with a rate of flow of 100 liters per minute. Pure carbon monoxid was used, introduced from a gasholder previously filled with carbon monoxid by the action of sulphuric acid on formic acid. The rates of flow and the concentrations used were controlled by flow meters.

A great divergency was noted in the behavior of the dogs, similar to that found by other observers, some dogs rapidly becoming unconscious, others showing a marked tolerance for the gas. In all cases animals were carried through the first period of rapid breathing and convulsions, then through the period of Cheyne-Stokes respiration to the point where slowing respiration indicated that the limit had been reached. Even under these conditions marked variations in the carbon monoxid hemoglobin content were found, and a number of animals were lost in spite of artificial respiration and treatment. Immediately on removal from the chamber a sample of blood was taken, and further samples were taken every ten minutes as noted in the tables. The percentage of hemo-

9. Briggs, Henry: Tr. Roy. Soc., Edinburgh, 1924.

10. This apparatus is described in Colliery Guardian **128**:310 (Aug. 1) 1924.

globin saturated with carbon monoxid was estimated by the pyrotannic method described by Sayers, Yant and Jones.[11]

The control animals were placed on cradles and allowed to breathe ordinary air. The treatments on the other subjects were carried out as follows: Large cylinders were filled with the different mixtures to be used, and after twenty-four hours (to allow diffusion) samples were drawn off and analyzed to insure the proper mixture. Reducing valves were attached to the cylinders and then to a rubber hose leading to large signal corps rubber balloons. From the balloons a short length of three-fourths inch (1.87 cm.) hose led to the dog face-mask. These were made by cutting off the bottoms of a number of various sized bottles to insure accurate fits; the cut end was padded and held tightly over the subject's face. The entering pipe was fitted with a check valve that prevented the exhaled air from reentering the breathing bags. A hole in the sides of the bottles contained a flutter valve, which kept the dog from inhaling air from the room. The balloons were kept well distended with the treatment mixture, so much so that there was a continual loss through the valves. This, while wasteful, assured us that the animal was receiving a sufficient supply of the mixture and was not rebreathing. The use of the smallest possible bottles as face-masks cut the factor of dead air space down to the minimum.

Ten dogs were run in each series. By adding the figures for the same time and averaging them it is possible to draw a curve that represents the average decline in carbon monoxid content of the blood under the various treatments tested. The averaging method gives the same results as several other methods that were tried and several statisticians who have examined the results state that the averaging method used is satisfactory.

PRESENTATION OF DATA

In the tables given here it is to be noted that different degrees of saturation of the blood were effected. This gives a general view of the results of treatment of cases of varied severity.

In Table 1 the individual variations in dogs without treatment is strikingly brought out and under precisely similar conditions some of the subjects eliminate carbon monoxid much faster than others.

Again the remarkable variation in rate of decrease is seen in Table 2. The average rate of decrease is of course much more rapid than in Table 1.

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11. Sayers, R. R.; Yant, W. P., and Jones, G. W.: The Pyrotannic Acid Method for the Quantitative Determination of Carbon Monoxid in the Blood, Pub. Health Rep. **38**:2311-2320 (Oct. 5) 1923.

TABLE 1.—*Rate of Elimination with No Treatment*

Experiment	Weight of Dog, Lb.	Concentration of Carbon Monoxid	Exposure in Min.	Percentage of Hemoglobin Combined with Carbon Monoxid, at Ten Minute Intervals from End of Exposure								Remarks
				0	10	20	30	40	50	60	70	
1	23 (10.4 kg.)	0.25	90	50	35	30	30	20	15	10	..	Respiration quiet and normal throughout
2	28 (12.7 kg.)	0.25	90	50	40	30	..	30	20	10	10	
3	26 (11.8 kg.)	0.25	105	50	50	45	40	40	35	25	..	
4	33 (15 kg.)	0.25	95	55	45	35	25	20	15	10	..	On removal from chamber panting; still unconscious at 60 minutes
5	33 (15 kg.)	0.25	88	65	47	40	35	35	30	30	..	
6	23 (10.4 kg.)	0.25	65	60	50	45	35	35	25	20	20	At 10 minutes, respiration 76 and shallow
7	25 (11.3 kg.)	0.25	80	55	50	45	30	25	..	..	..	
8	24 (10.9 kg.)	0.25	90	65	50	40	35	30	25	15	..	
9	22 (10 kg.)	0.25	80	55	50	50	45	40	40	30	..	
10	29 (13.2 kg.)	0.25	75	55	45	40	40	..	35	30	30	At 10 minutes, respiration shallow, 24 per minute
Average				56	46.2	40	35	30.5	26.6	20	20	

TABLE 2.—*Rate of Elimination with Oxygen Treatment* *

Experiment	Weight of Dog, Lb.	Concentration of Carbon Monoxid	Exposure in Min.	Percentage of Hemoglobin Combined with Carbon Monoxid, at Ten Minute Intervals from End of Exposure							Remarks
				0	10	20	30	40	50	60	
1	27 (12.2 kg.)	0.25	85	50	30	20	10	..	..	..	
2	28 (12.7 kg.)	0.25	90	70	45	35	30	20	10	5	Respiration 180 per minute at 10 minutes; after 50 minutes respiration 24
3	26 (11.8 kg.)	0.25	40	60	35	20	20	5	5	..	
4	24 (10.9 kg.)	0.25	75	60	45	20	..	10	5	..	Artificial respiration required at first
5	26 (11.8 kg.)	0.25	130	50	30	20	15	5	..	..	
6	19 (8.6 kg.)	0.25	80	70	40	30	10	5	..	..	Respiration 76 on removal from chamber
7	22 (10 kg.)	0.25	40	45	15	5	..	..	..	..	Artificial respiration at first; old dog
8	13 (5.9 kg.)	0.25	46	45	10	5	..	..	..	..	
9	18 (8.2 kg.)	0.25	110	65	45	30	20	15	10	..	Respiration at 0, 20 and deep
10	36 (16.3 kg.)	0.25	58	60	35	25	15	10	10	..	At 10 minutes, respiration 144, shallow
Average				57.5	33	21	17.1	10	8	5	

* Analysis of cylinders showed oxygen 98.5 per cent.

TABLE 3.—*Rate of Elimination with Oxygen Plus 5 Per Cent. Carbon Dioxid*

Experiment	Weight of Dog, Lb.	Concentration of Carbon Monoxid	Exposure in Min.	Percentage of Hemoglobin Combined with Carbon Monoxid, at Ten Minute Intervals from End of Exposure						Analysis	Remarks
				0	10	20	30	40	50		
1	24 (10.9 kg.)	0.25	90	45	20	5	..	..	..	CO_2= 5.8 O_2 =92.8	Respiration between 70 and 80 per minute
2	25 (11.3 kg.)	0.25	110	50	25	10	2	..	..	CO_2= 5.6 O_2 =93.6	Respiration immediately increased when treatment began
3	20 (9 kg.)	0.25	55	50	25	10	..	..	..	CO_2= 5.6 O_2 =93.6	
4	24 (10.9 kg.)	0.25	80	50	20	20	15	5	..	CO_2= 5.0 O_2 =93.6	Artificial respiration at first
5	20 (9 kg.)	0.25	105	60	25	10	5	..	..	CO_2= 5.0 O_2 =93.8	
6	22 (10 kg.)	0.25	84	60	35	20	10	..	..	CO_2= 5.0 O_2 =93.8	
7	33 (15 kg.)	0.25	70	60	30	15	10	5	..	CO_2= 5.8 O_2 =92.8	Fairly deep respiration
8	24 (10.9 kg.)	0.25	80	55	30	15	10	5	..	CO_2= 5.2 O_2 =94.1	At 20 minutes, respiration 60, fairly deep
9	24 (10.9 kg.)	0.25	53	65+	40	25	20	15	5	CO_2= 4.7 O_2 =94.5	At 10 minutes, respiration 82, deep; artificial needed at 0
10	28 (12.7 kg.)	0.25	69	60	35	20	15	..	7	CO_2= 5.8 O_2 =92.8	At 10 minutes, respiration rapid and fairly deep
Average.....				55.5	28.5	15	10.8	7.5	6		

TABLE 4.—*Rate of Elimination with Oxygen Plus 10 Per Cent. Carbon Dioxid*

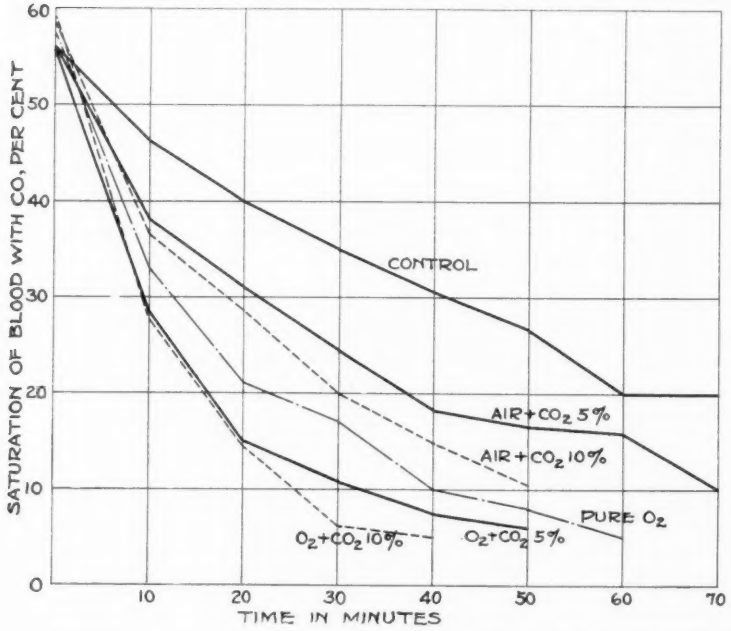
Experiment	Weight of Dog, Lb.	Concentration of Carbon Monoxid	Exposure in Min.	Percentage of Hemoglobin Combined with Carbon Monoxid, at Ten Minute Intervals from End of Exposure						Analysis	Remarks
				0	10	20	30	40	50		
1	17 (7.7 kg.)	0.25	60	60	20	10	5	..	..	CO_2=10.0 O_2 =87.2	At 0 minute, respiration 96; at 10 minutes, 120, very deep
2	34 (15.4 kg.)	0.25	75	50	25	20	5	..	..	CO_2=10.0 O_2 =87.2	At 0 minute, respiration 140; at 10 minutes, respiration 154
3	23 (10.4 kg.)	0.25	61	65	27	20	10	..	..	CO_2= 9.6 O_2 =89.4	Breathing very hard, fast and deep
4	26 (11.8 kg.)	0.25	75	60	25	10	5	..	..	CO_2=10.0 O_2 =89.2	Same as Dog 3
5	26 (11.8 kg.)	0.25	72	65	30	15	5	..	..	CO_2= 9.6 O_2 =89.4	At 10 minutes, respiration very deep; artificial respiration at first
6	27 (12.2 kg.)	0.25	70	60	30	15	5	..	..	CO_2= 9.6 O_2 =89.4	Respirations deep and fast
7	33 (15 kg.)	0.25	85	65	30	20	10	5	..	CO_2=10.0 O_2 =89.2	At 10 minutes, respiration 80, very deep and full
8	16 (7.3 kg.)	0.25	85	50	40 (Second sample poor)	10	7	..	..	CO_2=10.1 O_2 =88.5	Respiration very deep
9	16 (7.3 kg.)	0.25	90	60	30	15	5	..	..	CO_2=10.0 O_2 =89.2	Respirations deep and rapid
10	17 (7.7 kg.)	0.25	53	55	20	10	5	..	..	CO_2=10.0 O_2 =89.2	Respiration at 10 minutes, 60, very deep
Average.....				59	27.7	14.5	6.2	5			

In the series in Table 3 the rate of fall was slightly greater than in the oxygen series. The respiration of these animals was fairly deep, but no more so than that of some of the dogs treated with plain oxygen.

In the series in Table 4 the rate of elimination was even more rapid. The same individual variation in dogs was to be noted. The breathing of the animals was deep and rapid, the muscular effort involved being great.

The respiration in this series of animals, while fairly rapid and deep was less so than in the animals treated with the other mixtures.

The respirations of the animals in Table 6 was very deep and rapid.



Rapidity of fall of carbon dioxid content in blood under different treatments.

DISCUSSION OF DATA

No variation was noted in the after effects of the different treatments. There was no increased tendency toward paralysis in any particular group, and it would be impossible to tell from the after history of the dogs what treatment they had received.

The individual idiosyncrasy of dogs to the gas is marked. Under similar conditions some dogs were degassed much more rapidly than others in the same group.

TABLE 5.—*Rate of Elimination with Air Plus 5 Per Cent. Carbon Dioxid*

Experiment	Weight of Dog, Lb.	Concentration of Carbon Monoxid	Exposure in Min.	Percentage of Hemoglobin Combined with Carbon Monoxid, at Ten Minute Intervals from End of Exposure								Analysis	Remarks
				0	10	20	30	40	50	60	70		
1	25 (11.3 kg.)	0.25	80	70	50	50	40	35	30	25	20	CO_2= 5.0 O_2 =20.2	
2	31 (14.1 kg.)	0.25	90	50	40	35	30	20	15	0	..	CO_2= 5.1 O_2 =19.7	Respirations dropped from 70 to 60; always deep
3	29 (13.2 kg.)	0.25	70	55	35	30	20	10	0	..	..	CO_2= 5.1 O_2 =19.7	Respiration remained between 32 and 36
4	25 (11.3 kg.)	0.25	78	55	25	20	15	10	8	0	..	CO_2= 4.8 O_2 =21	At 10 minutes, respiration 48 and deep
5	39 (17.7 kg.)	0.25	80	62	40	30	25	20	20	15	10	CO_2= 4.8 O_2 =21	At 20 minutes, respiration 160, deep; at 40, respiration 100, fairly deep
6	24 (10.9 kg.)	0.25	80	60	45	40	30	20	15	15	10	CO_2= 4.9 O_2 =20	At 10 minutes, 80, fairly deep
7	20 (9 kg.)	0.25	56	55	40	30	25	20	15	10	10	CO_2= 5.2 O_2 =18	At 10 minutes, 44, deep
8	38 (17.2 kg.)	0.25	65	55	45	35	35	25	20	20	10	CO_2= 5.2 O_2 =18	
9	22 (10 kg.)	0.25	84	50	30	20	15	..	10	10	..	CO_2= 4.8 O_2 =20	At 10 minutes, respiration 56, deep
10	18 (8.2 kg.)	0.25	83	47	30	20	10	5	0	0	..	CO_2= 4.8 O_2 =20	At 10 minutes, respiration 80, fairly deep
Average				55.9	38	31	24.5	18.3	16.6	15.8	10		

TABLE 6.—*Rate of Elimination with Air Plus 10 Per Cent. Carbon Dioxid*

Experiment	Weight of Dog, Lb.	Concentration of Carbon Monoxid	Exposure in Min.	Percentage of Hemoglobin Combined with Carbon Monoxid, at Ten Minute Intervals from End of Exposure						Analysis	Remarks
				0	10	20	30	40	50		
1	25 (11.3 kg.)	0.25	70	50	35	25	15	10	..	CO_2= 9.3 O_2 =19.2	Respirations 60 per minute, deep and violent
2	24 (10.9 kg.)	0.25	45	70	40	35	25	20	10	CO_2= 9.2 O_2 =19.2	Respirations 128; deep at 10 minutes; artificial respiration necessary
3	29 (13.2 kg.)	0.25	63	55	35	30	20	15	10	CO_2= 9.3 O_2 =19.2	At 0 minute, respirations 66, fairly deep
4	40 (18.1 kg.)	0.25	94	70	45	35	30	20	15	CO_2=10.2 O_2 =18	At 10 minutes, respirations 100; very deep; at 40 minutes, deep
5	19 (8.6 kg.)	0.25	100	60	40	30	25	20	15	CO_2=10.0 O_2 =20.1	At 10 minutes, respirations 70, very deep
6	19 (8.6 kg.)	0.25	64	55	35	25	15	15	5	CO_2=10.0 O_2 =20.1	At 10 minutes, respirations 80, deep
7	22 (10 kg.)	0.25	71	50	30	20	15	10	10	CO_2= 9.5 O_2 =20	
8	22 (10 kg.)	0.25	52	55	35	25	15	10	10	CO_2=10.0 O_2 =20.1	At 10 minutes, respirations 60, very deep
9	34 (15.4 kg.)	0.25	80	60	35	30	15	15	10	CO_2=10.6 O_2 =19.8	At 10 minutes, respirations 60, very deep; at 30 minutes, respirations 48, very deep
10	25 (11.3 kg.)	0.25	96	60	35	30	25	15	10	CO_2= 9.5 O_2 =20	At 10 minutes, respirations 80, very deep; at 30 minutes, respirations 80, very deep
Average				58.5	36.5	28.5	20	15	10.5		

Our findings confirm the statement of Sayers [4] that pure oxygen gives almost as rapid elimination of carbon monoxid from the blood as does the oxygen plus a 5 per cent. carbon dioxid mixture. Our findings do not confirm the statement of Henderson [1] and Haldane [5] that air plus 5 per cent. carbon dioxid washes out carbon monoxid more rapidly than pure oxygen. We agree with Nicloux, Nerson, Stahl and Weill [7] in their statement that oxygen washes out carbon monoxid more rapidly than a mixture of air and carbon dioxid, either 5 or 10 per cent.

In carbon monoxid asphyxia the period of danger is when the blood is over 30 per cent. saturated with the gas. Any treatment that safely and rapidly reduces the blood saturation below this point is of value. The tables show that the rapidity of reduction by the treatments considered comes in the following order: oxygen plus 10 per cent. carbon dioxid, oxygen plus 5 per cent. carbon dioxid, oxygen, air plus 10 per cent. carbon dioxid, air plus 5 per cent. carbon dioxid.

Of these five treatments, two, the oxygen plus 10 per cent. carbon dioxid and the air plus 10 per cent. carbon dioxid can be dismissed at once, for there is a possible danger in their use since a weakened or diseased heart might be overtaxed by the violent respiratory efforts induced by them.

If a reduction to 25 per cent. saturation of the blood be taken as a desirable point to reach as quickly as possible, an examination of the accompanying chart shows that oxygen plus 5 per cent. carbon dioxid dogs reach this point in twelve and one-half minutes, oxygen dogs in sixteen and one-half minutes and air plus 5 per cent. carbon dioxid dogs in twenty-nine minutes.

According to this standard, while there is but little difference in the value of the oxygen treatment and the oxygen plus 5 per cent. carbon dioxid treatment, both are much superior to the air plus 5 per cent. carbon dioxid treatment.

If any other point of saturation be selected as the goal to be reached as rapidly as possible, consultation of the chart will give the relative value of the treatments as regards the time required.

As compared to the untreated dogs the chart shows that all the treatments considered give a rapid rate of reduction of the carbon monoxid and a more rapid arrival of the blood at a point of saturation where any further injury to the central nervous system is not to be feared.

CONCLUSIONS

1. In dogs suffering from carbon monoxid asphyxia the difference in elimination time between oxygen treated and oxygen plus 5 per cent. carbon dioxid treated subjects is slight.

2. Since the rate of elimination under pure oxygen is almost as rapid as with the oxygen plus 5 per cent. carbon dioxid and since the oxygen treatment is more readily available it is recommended that its use be continued, particularly in localities where transportation is difficult.

3. The air plus 5 per cent. carbon dioxid treatment should be of value for rescue parties sent to inaccessible mines and especially in military operations.

THE ENZYMIC ACTIVITY OF THE DUODENAL CONTENTS FOLLOWING THE INGESTION OF PANCREATIN *

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The purpose of this article is to demonstrate that a pancreatin containing the three active ferments trypsin, steapsin and amylopsin, properly administered by mouth, on reaching the duodenum will retain a considerable proportion of its original enzymic activity.

Long [1] has shown experimentally on dogs with Pawlow pouches that the amylopsin of a pancreatic extract is quickly reduced in activity or destroyed by the hydrochloric acid of the gastric secretion. He also noted that trypsin retained a large percentage of its activity if protected by food proteins from the digestive action of the hydrochloric acid-pepsin combination. From this he concluded that trypsin, thus protected, would unquestionably reach the duodenum and there exert its specific digestive action. There are no available studies as to the fate of lipase administered by mouth.

The studies of Long did not include an examination of the duodenal contents for pancreatic extract that had passed on into the duodenum after having been in the stomach and exposed to the action of gastric juice. He found that the retention of enzymic activity by pancreatic extract exposed to gastric secretion was inversely proportional to the length of exposure and to the p_H of the gastric juice; also that if large amounts of pancreatin were used, thereby increasing the concentration of the ferments, destruction was much less complete.

Stimulated by his work we will endeavor to show that pancreatin given in large amounts and in proper solution will pass through the stomach rapidly enough to avoid the destructive influence of the gastric juice and reach the duodenum to a large extent undestroyed.

The enzymic activities in the following experiments were determined by McClure's [2] methods, slightly modified, as these have been

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1. Long, J. H., and Muhleman, G. W.: Arch. Int. Med. **13**:314 (Feb.) 1914. Long, J. H., and Hull, Mary: J. Am. Chem. Soc. **38**:1620, 1916; **39**:162, 1917.

2. McClure, C. W.; Wetmore, A. S., and Reynolds, L.: New Methods for Estimating Enzymatic Activities of Duodenal Contents of Normal Man, Arch. Int. Med. **27**:706 (June) 1921.

recognized as the most accurate and reliable that are available. Tryptic activity is expressed as milligrams of nonprotein nitrogen resulting from the digestive action of the specimen tested on a buffered casein solution. Lipolytic activity is expressed as the number of cubic centimeters of twenty-fifth normal sodium hydroxid solution necessary to neutralize the acid resulting from the digestive action of the specimen tested on a 5 per cent. aqueous solution of glyceryl triacetate. Glyceryl triacetate was substituted for the cotton seed oil emulsion of McClure's method. Amylolytic activity is expressed as milligrams of glucose resulting from the digestive action of the specimen tested on a 2 per cent. buffered starch solution.

The pancreatin solution used in our experiments was prepared by mixing 10 gm. of pancreatin, U. S. P., with 200 c.c. of water at 10 degrees C. The three enzymes quickly pass into colloidal solution and a grayish, cloudy mixture results. This we have designated a 5 per cent. pancreatin solution. It was freshly prepared and its enzymic activities determined in each experiment. Our figures show that this solution possessed approximately the same tryptic and lipolytic action as duodenal contents while its amylolytic activity was three times greater. Cold water at 10 degrees C. was used in preparing the pancreatin solution as it has been shown that cold protects the enzymes from the destructive action of the gastric juice.

PROCEDURE

The duodenal tube was swallowed by a fasting subject and the tip located in the second portion of the duodenum. A specimen of fasting duodenal contents was aspirated and its enzymic activity determined. After this specimen was secured 200 c.c. of cold water was administered by mouth and a duodenal drip was established which persisted about fifteen minutes in each case. Specimens of this watery drip were secured at various intervals and their enzymic activities determined.

After the lapse of an hour 200 c.c. of the specially prepared 5 per cent. pancreatin solution described above was administered orally, and in from two to five minutes produced a duodenal drip possessing all the physical characteristics of the colloidal solution ingested. In the greater number of our cases 75 per cent. of the 5 per cent. pancreatin solution could be recovered from the duodenum in ten minutes. Specimens of this duodenal drip were collected at one or two minute intervals until the characteristic colloidal solution could no longer be secured.

The enzymic activities of these specimens were determined and compared with the activities of the specimens secured following the ingestion of cold water. This procedure was followed as closely as possible in ten cases. Twice we administered the 5 per cent. pancreatin solution forty-five minutes after a meal consisting of an egg, bread,

butter and milk; a duodenal drip again appeared and the enzymic activities of this drip were determined.

The charts given here explain the results of our experimentation. Chart 1 shows the enzymic activities of specimens collected after water had been administered orally, demonstrating the low activities due to water dilution. Chart 2 contrasts the activities of the specimens recov-

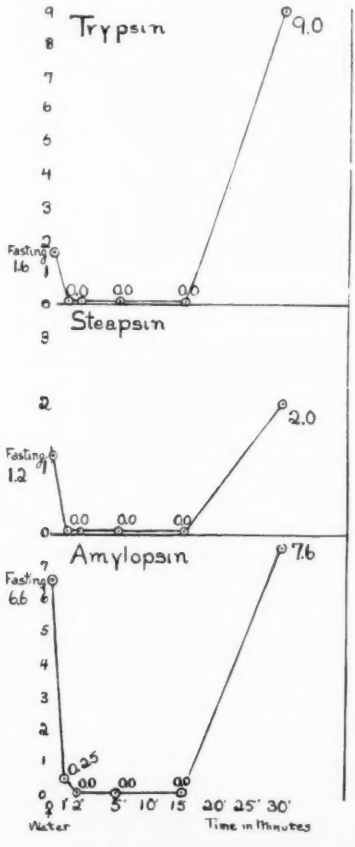

Chart 1.—Typical water dilution curve showing reduction in enzymic activities over a period of fifteen minutes following the ingestion of 200 c.c. of cold water.

ered from the duodenum after water and after 5 per cent. pancreatin. Chart 3 is a composite chart of our twelve cases. Each figure plotted on this chart represents an average of from six to twelve enzyme determinations. The enzymic activities of all the specimens we collected in our experimentation were grouped according to time intervals

after the ingestion of either the water or the 5 per cent. pancreatin and an average figure calculated for each time period. The contrast between the curves on this chart is striking and presents reliable evidence that large quantities of the enzymes reached the duodenum after the ingestion of the 5 per cent. pancreatin solution. It shows also that the destruction of trypsin is less rapid and less complete than that of steapsin and amylopsin.

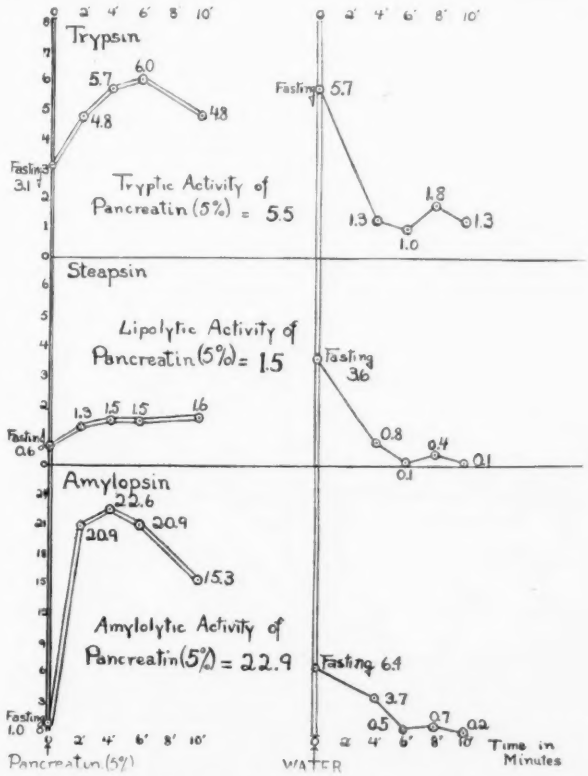

Chart 2.—Water dilution curve over a period of ten minutes and curve following ingestion of 5 per cent. pancreatin solution; marked rise of enzymic values following pancreatin and drop after water. The nine other cases studied in a similar manner showed the same phenomena.

SUMMARY AND CONCLUSIONS

It is possible to administer a pancreatin solution orally which will pass through the stomach and can be recovered from the duodenum by means of the duodenal tube. That this is the solution that has been administered orally is demonstrated by the fact that the specimens

collected from the duodenum possessed all the physical characteristics of that solution. We found that the 5 per cent. pancreatin solution could be administered orally and portions of it could be recovered from the duodenum in from two to five minutes. Enzymic activities on specimens recovered at various times after pancreatin were much greater than those recovered in a similar manner after the ingestion of water.

The gastric contents of all the subjects studied contained free hydrochloric acid in various amounts and it was noted that in those that

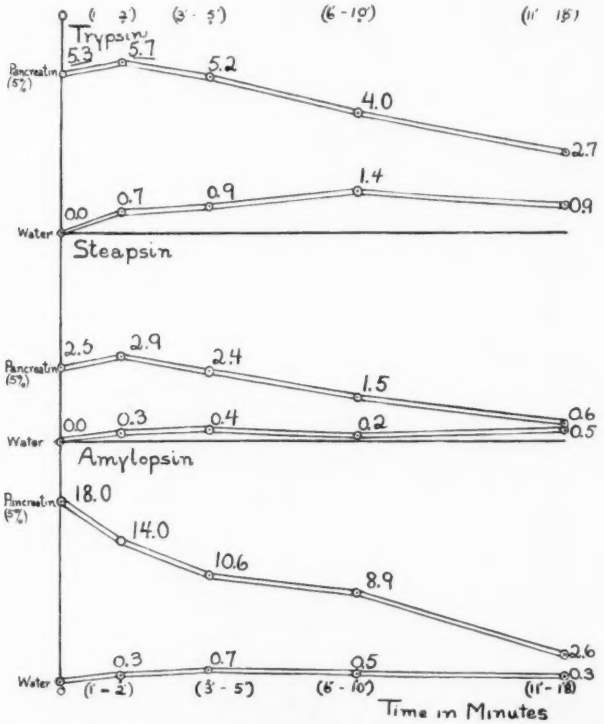

Chart 3.—Water and pancreatin curves in twelve cases studied; the enzymic activities of all the specimens collected during experimentation were grouped into time intervals following the ingestion of either water or 5 per cent. pancreatin solution and an average figure for each time period calculated.

tended toward hyperchlorhydria the enzymic activities were more rapidly reduced than in cases of subacidity.

We believe that it will be necessary to administer pancreatic extract containing the three ferments in cold water and in larger dosage than has been used previously, to insure its entrance into the duodenum

undestroyed. This can be accomplished by administering 5 gm. of pancreatin suspended in cold water either on an empty stomach or forty-five minutes after a meal. The latter fact was determined by recovering the pancreatin solution from the duodenum forty-five minutes after a meal and finding high enzymic activity therein.

From our studies trypsin is least affected of the three ferments by the gastric juice, which agrees with Long's experimental findings. We have also found that amylopsin and steapsin reach the duodenum to a large extent undestroyed, this being rendered possible by the rapidity with which they traverse the stomach. It is difficult to estimate what portion of the enzymes thus administered do reach the duodenum undestroyed, probably most all of the trypsin and from 50 to 60 per cent. of the amylopsin and steapsin in the average case.

No conclusive study of the clinical effects of enzymes thus orally administered has been made and no opinion will be expressed on this problem. However, we conclude from our experiments that a properly prepared and ingested pancreatin solution will pass through the stomach rapidly enough to insure the retention of a considerable portion of its original proteolytic, lipolytic and amylolytic activity when it reaches the duodenum.

THE RELATION OF THROMBOCYTES TO HEMORRHAGIC DIATHESIS

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The clinical study of thrombocytes is rapidly gaining recognition not only in diseases connected with the hematopoietic system but also in infectious and metabolic disorders. Stahl [1] and others have recently pointed out the importance that the number, the size and the staining of the thrombocytes play in the diagnosis and prognosis of various diseases. This discussion will be confined chiefly to the rôle played by the thrombocytes in hemorrhagic diathesis.

For a long time the thrombocytes had been considered as imperfect or deformed red cells, that is, the débris of red blood cells. In 1877 Hayem described thrombocytes under the name of hematoblasts because he believed these structures to be youthful forms of red blood cells. In 1881 Brohm observed two cases of Werlhoff's disease and was the first to point out that in this affection the hematoblasts were markedly diminished.

In 1883 Krauss,[2] in the clinic of Brohm in his dissertation on Werlhoff's disease, pointed out that the hematoblasts are markedly diminished during the height of the disease and that they reappear when the hemorrhages disappear. If Krauss had been familiar with the contribution of Bizzozero,[3] who a year previous described hematoblasts under the name of blood platelets and designated them as a third independent formed element of the blood, and stated that the aggregation of the blood platelets is the underlying foundation of every thrombus, Krauss would have correlated more closely the bleeding with the diminution of blood platelets. Brohm attributed the disease to a breaking up of the hematoblasts and their deleterious effect on the blood vessels. Denys,[4] the Belgian pathologist, working independently found that the blood platelets are diminished in certain diseases accompanied by hemorrhage. It remained for Duke [5] in this country in 1910 to point out emphatically and conclusively that the hemorrhages in Werlhoff's purpura are due to a diminution of the blood platelets. Based on extensive clinical material and experimental studies that consisted in diminishing the number of blood platelets in the rabbit by injections of diphtheria antitoxin or

1. Stahl, R.: Ztschr. f. ang. Anat. Festschrift fur Martius **6**:301-319, 1920; Ztschr. f. klin. Med. **96**:182-211 (Jan.) 1923.
2. Krauss, E.: Ueber Purpura, Inaugural Dissertation, Heidelberg, 1883.
3. Bizzozero: Virchows Arch. f. path. Anat. **90**:251, 1882.
4. Denys: Zentralbl. f. allg. Path. u. path. Anat., 1893.
5. Duke, W. W.: J. A. M. A. **55**:1185 (Oct. 1) 1910; J. Exper. Med. **14**: 265, 1911; Arch. Int. Med. **10**:445 (Nov.) 1912.

benzene, he found that if the blood platelets were diminished below 35,000 to the cubic millimeter, hemorrhages resulted which disappeared after they increased in number. The blood platelets also can be made to disappear from the blood by injections of peptone, gelatin and colloid. Even if they do not disappear after the injection of such substances, they lose their thrombus forming ability. Lee and Robertson [6] caused a diminution of blood platelets in rabbits by injections of antiplatelet serum. Duke emphasized the difference between the coagulation time and the bleeding time and proved that the prolonged bleeding time is caused by a diminution in blood platelets. Hayem recognized the fact that the retractability of the blood clot is influenced by the blood platelets. In fact the recent studies of Frank,[7] Kaznelson,[8] Keisman,[9] Brill and Rosenthal [10] are due to the stimulating and brilliant work of Duke.

It is now a universally accepted fact that the blood platelets are independent elements of the blood. Normally, they number 300,000 or 350,000 to the cubic millimeter if counted according to the method of Hayem, but according to Pratt's [11] method, they total 469,000 to the cubic millimeter. They are uniform in size, from 2 to 4 microns in diameter. Wright [12] showed that they are derived from the megakaryocytes of the bone marrow, that they reach the circulating blood by budding off from the mother cell, and that they possess ameboid movement. They never leave the blood stream as is the case with the red and white blood cells. This explains why the blood platelets are never found in lymph, serous cavities or purulent collections. They are at times found in large numbers in the spleen, where they are normally destroyed. If the blood is allowed to flow from blood vessels the blood platelets break down readily. This is generally attributed to the fact that they lose their carbon dioxid. They are the only elements in the blood in which thrombogen has been demonstrated; this accounts for its property of thrombus formation. Frank terms the blood platelets thrombocytes because of this ability. Duke as well as Minot [13] and Frank demonstrated conclusively that if the thrombocytes in the blood are reduced below 50,000 even slight trauma causes ecchymosis. On this fact is based the capillary stasis test (Rumpel-Leede), also known as the capillary resistance test.[14]

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6. Lee, R. I., and Robertson, O. H.: J. M. Res. **33**:323 (Jan.) 1916.
7. Frank, E.: Ergebn. d. ges. Med. **3**:171-211, 1922.
8. Kaznelson, P.: Ztschr. f. klin. Med. **87**:88.
9. Keisman, M.: Med. Klin., January, 1922, No. 3.
10. Brill, N. E., and Rosenthal, N.: Am. J. M. Sc. **166**:503 (Dec.) 1923; Arch. Int. Med. **32**:939-953 (Dec.) 1923.
11. Pratt, J. H.: Osler and McRae Modern Medicine **4**:694, 1915.
12. Wright, J. H.: Boston M. & S. J. **154**:643, 1906; Virchows Arch. f. path. Anat. **186**:55, 1906.
13. Minot: Am. J. M. Sc. **152**:48 (July) 1916.
14. Hess, A. F.: Hemophilia, Arch. Int. Med. **17**:203 (Feb.) 1910.

According to Bordet,[15] the thrombocytes furnish a cytozyme that causes the formation of a blood clot. Stahl demonstrated that morphologically two types of thrombocytes may be encountered even in healthy persons, namely, the neutrophilic giant thrombocyte and the basophilic giant thrombocyte; the former once found is always present, the latter is only occasionally encountered and is more frequent in disease. They are the direct consequence of irritation of the megakaryocytes and are, in reality, the immature or youthful thrombocytes. These immature forms may sometimes be small or of medium size.

A slowing up of the blood stream causes the thrombocytes to adhere to the zone of the vessels and is thus responsible for thrombosis intra vitam in cases of circulatory weakness. Wherever there is a foreign body in the tissues, the thrombocytes collect around it. They have an agglutinative property by virtue of which they rapidly adhere to cut or injured surfaces of tissues; this results in the formation of a thrombus. The minimum number of thrombocytes necessary for this adhesive property is low compared to the number of blood platelets encountered in the essential thrombocytopenic state. Their agglutinative power also explains the retractile property of the blood clot and the presence of a nonretractile blood clot if the thrombocytes are greatly diminished. They have neither nuclei nor definite shape. It was recently demonstrated why they increase in number, size and in basophilic granules in many infectious diseases. It has also been shown by Onaka that they consume much oxygen.

Hemorrhagic diathesis characterized by a marked reduction of thrombocytes in the blood has been termed by Frank thrombopenia and by Eppinger thrombocytopenia. In view of the unknown etiology of this disease, it is termed essential thrombopenia or essential thrombocytopenia.

Thrombocytopenia has clinical manifestations similar to hemophilia except that in hemophilia the coagulation time is prolonged and the bleeding is never spontaneous. In hemophilia, cytozyme is diminished according to Bordet, hence the delay in coagulation time. In thrombocytopenia, cytozyme is normal. In essential thrombocytopenia, cytozyme is rapidly formed, but the amount is diminished on account of the presence of fewer thrombocytes, which cause prolonged bleeding. The citrated clear purpuric plasma does not clot even after standing several hours, undoubtedly owing to the sedimentation of the thrombocytes.

An increase in thrombocytes is present in few diseases, such as chlorosis, secondary anemia, polycythemia rubra, megalosplenica (Vaquez) and, at times, in lymphatic leukemia.

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15. Bordet, J.: Bull. Johns Hopkins Hosp. **32**:213 (July) 1921.

CLINICAL ASPECTS: WERLHOFF'S DISEASE

In 1740 Werlhoff described a symptom complex under the name morbus maculosus hemorrhagica characterized by spontaneous hemorrhages from the mucous membranes and by the appearance of petechiae or ecchymosis under the skin. He states that the disease may occur in both sexes during all periods of life, but is somewhat more frequent in early adult life. It may run a course of varying severity but as a rule has no tendency to recur. At times the hemorrhages from the internal organs are so severe as to cause marked secondary anemia, so that a fatal outcome may seem inevitable when sudden recession of the symptoms with subsequent complete recovery occurs. A fatal termination is rare. In some cases the hemorrhages occur only from the internal organs, the gastro-intestinal tract, or from the kidneys and, more rarely, in the form of pulmonary hemorrhages. Hemorrhages into the pleura and pericardium may take place. Hemorrhages into the peritoneum are exceedingly rare. The disease runs an afebrile course or, at most, has a moderate elevation of temperature. Sometimes the temperature runs fairly high due to the absorption of blood from the hemorrhagic areas or to a secondary infection. The secondary infection may take place from the mouth, especially from the gums and the tonsils, already devitalized and ulcerated owing to the excessive loss of blood. The disease is neither constitutional nor hereditary, nor does it generally affect more than one member of the same family.

The symptomatology as described by Werlhoff can hardly be modified. A number of important factors have been added to the study of this disease. It has been proved that one of its main characteristics is the primary reduction in the number of thrombocytes, hence the name, essential thrombopenia (Frank) or essential thrombocytopenia (Eppinger). Fonio's [16] method for blood platelet count is advocated. The finger tip is cleansed with alcohol; then a drop of 14 per cent. magnesium sulphate solution is placed on it. A drop of blood is obtained from the finger tip covered with the applied solution. This causes the drop of blood to mix with the magnesium sulphate. A smear is then made and stained deeply with Giemsa solution. The thrombocytes appear isolated; both the erythrocytes and the blood platelets are counted. Only 1,000 red cells are counted and the number of erythrocytes to the cubic millimeter is determined. It is simple to calculate the number of thrombocytes. There is one thrombocyte to fifteen or twenty erythrocytes.

The prolongation of bleeding time is another important diagnostic sign in thrombocytopenia and is carried out as follows according to

16. Fonio: Cor.-Bl. f. schweiz. Aerzte. **45**:1505, 1915.

Duke's method: The lobe of the ear is punctured. Normally, the blood oozes from one to three minutes. In thrombocytopenia it is prolonged as long as ten minutes or even for hours.

The decrease in the number of thrombocytes is also responsible for the diagnostic sign described by Hayem, namely, the failure of the clot to retract. Glanzman [17] attributed this failure to some defect of the thrombocytes and called the condition thrombo-asthenia. Brill and Rosenthal pointed out that in thrombocytopenia before splenectomy, even if the thrombocytes increase to 80,000, the clot fails to retract; according to Brill and Rosenthal this signifies that the spleen in this disease counteracts the retractability of the blood clot. The coagulation time is usually not interfered with in this disease.

Another important diagnostic sign is the capillary resistance test (Rumpel-Leede; A. F. Hess). This depends both on the diminution of thrombocytes and the associated condition of the blood vessels in this disease. A tourniquet is applied to the arm from one to four minutes, tight enough to prevent the return circulation without obliterating the pulse. According to the degree of hemorrhagic diathesis, petechiae or even large ecchymotic areas appear on the forearm. The tourniquet should not be applied longer than five minutes because in that case petechiae would occur even in normal persons.

CLINICAL SUBDIVISIONS

According to the severity of the disease and according to its cause, Frank subdivides essential thrombocytopenia into benign and malignant forms. The benign form runs a clinical course corresponding to the mild type described by Werlhoff. The blood picture shows a reduction in the number of thrombocytes. This benign form may recur and cause a moderate degree of secondary anemia of the chlorosis type, in which the hemoglobin may be reduced out of proportion to the red blood cells. The latter show considerable vacuolization and moderate anisocytosis and poikilocytosis. The leukocytes are unaltered except that sometimes the mononuclears predominate or, occasionally, a few immature white cells are found. With the subsidence of the symptoms the blood picture rapidly returns to normal. The thrombocytes never return to their normal number. If the recurrent type of thrombocytopenia is continued over a long period of time, it may bring about a condition simulating hemophilia in which petechiae, ecchymosis and mild mucous membrane hemorrhages either are spontaneous or appear on slight trauma. This condition Frank terms pseudohemophilia. The coagulation time is delayed, but not to the same degree as in true hemophilia, which occurs exclusively, as is well known, in the male.

17. Glanzman: Jahrb. f. Kinderh. **88**:1-113, 1918.

Cases of chronic recurrent thrombocytopenia with the manifestations of pseudohemophilia must be differentiated from true hemophilia. True hemophilia is a hereditary abnormality of the blood or hematopoietic organs limited to males but transmitted by the females. It is characterized by a deficiency of prothrombin and cytozyme (Bordet) in the blood which causes a delay in coagulation and exposes the patient to fatal hemorrhage. It usually occurs much earlier in life than thrombocytopenia. It is often encountered in the first year of life. It is interesting to know that the umbilicus at birth is rarely a site of hemorrhage in hemophilia. Circumcision, on the other hand, may be the cause of fatal hemorrhage. In fact, piercing of the ears, snipping of the frenum of the tongue or circumcision may for the first time reveal the presence of the disease. The hemorrhages, wherever they occur, are preceded by trauma. The tendency to bleed varies in intensity at different times according to the state of coagulability of the blood at that particular time. At one time a slight trauma may result in severe hemorrhage, while at another time, even a severe trauma, may only cause slight ecchymosis. In the severe cases the joints are markedly affected, and if so, the hemorrhages usually reach the periosteum and the bone proper with consequent hemorrhagic periosteitis and osteitis. According to the studies of McLean,[18] the anticoagulation action of the blood in this disease has been experimentally demonstrated to be due to the action of the two phosphatids, one of which is isolated from the heart muscle and the other from the liver. If these phosphatids are added to normal blood in vitro or in vivo, a decrease in the amount of prothrombin results together with an increase in the antithrombin. The injection of phosphatid into the circulation of dogs causes hemophilic manifestations. According to Eddis,[19] there is a qualitative defect in the prothrombin in this disease. The important differentiating points between hemophilia and thrombocytopenia are: The former occurs exclusively in males with characteristic hemorrhages into the joints; it is hereditary; it has a delay in coagulation time and a delay in prothrombin time, a normal number of thrombocytes and a normal bleeding time with the normal retractabile blood clot.

The differential diagnosis of scurvy may at times come into consideration. This disease as it is now universally accepted is primarily a deficiency disease and the symptoms are the result of avitaminosis. If food, such as green vegetables, fruit juices, potatoes and raw milk, is added to the diet of those afflicted with scurvy, the symptoms rapidly disappear. In this affection the hemorrhages are more marked in the gums and around the hair follicles. In adults they are more marked in the muscles of the calf, and in children the joints (Moller-Barlow's

18. McLean, Jay: Am. J. Physiol. **41**:250 (Aug.) 1916.
19. Eddis: Proc. Soc. Exper. Biol. & Med. **14**:19, 1916-1917.

disease) are more often affected. The coagulation time and bleeding time are not interfered with and the thrombocytes are normal in number. According to Matthes,[20] the skin in scurvy has a grater-like roughness and the gums are not as frequently affected as is stated by Littens. The disease is often preceded by rheumatic pains, general malaise and pain in the gums. If the disease is recognized during this stage and proper treatment instituted, the hemorrhages into the gums do not occur. Bleeding from the gums occurs only where the teeth are present. The same author also states that during the World War, hemorrhagic effusion into the pleura was frequently encountered in scurvy. Selle and Rosenberg,[21] who contributed an excellent treatise on scurvy, state that adults afflicted with scurvy have a tendency to walk on the tips of the toes and present the jumping-jack phenomenon.

MALIGNANT TYPE OF THROMBOCYTOPENIA

The malignant type is that in which the hemorrhages from the internal organs with marked ulceration of the mouth, tonsils and pharynx occur; the petechiae are less marked. The anemia is marked. Hemoglobin may be as low as 30 per cent., and in some cases as low even as 20 per cent. Not only are the thrombocytes reduced to a minimum and, sometimes, even completely absent, but the red cells are considerably reduced in number. They show marked poikilocytosis and anisocytosis and Howell-Jolly bodies as well as Cabot's rings—a sign of marked destruction of the red blood cells. Leukopenia as low as from 1,500 to 2,000 to the cubic millimeter, with a diminution or complete disappearance of neutrophils, a replacement of neutrophils with lymphocytes and also the disappearance of eosinophils is encountered. In malignant thrombocytopenia not only are the megakaryocytes affected but the erythropoietic and myelocytic element of the bone marrow as well. Because of the leukopenia Frank terms this condition aleukia. This term does not seem justifiable because it signifies only that the leukocytes are diminished while in reality the red blood cells and thrombocytes are equally altered. Furthermore, the term aleukia often leads to a confusion of the condition with subleukemic or aleukemic leukemia, diseases from which it must be carefully differentiated, from both the prognostic and the therapeutic standpoints. It is therefore much more advisable to adhere to the term malignant thrombocytopenia. The term malignant does not signify that the condition is invariably fatal, for patients have recovered spontaneously or after appropriate treatment. It is malignant from the standpoint of symptomatology.

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20. Matthes: Differential Diagnosis, translated by Held and Gross, Philadelphia, 1925, pp. 114 and 327.

21. Selle and Rosenberg: Ergebn. d. inn. Med. u. Kinderh. **19**, 1921.

Malignant thrombocytopenia must be differentiated from the following conditions: subleukemic and aleukemic leukemia, of both the lymphatic and the myeloblastic types, sepsis and agranulocytic angina.

The acute type of aleukemic leukemia of the lymphatic types sets in with high temperature, ulceration of the gums and pharynx, and necrosis of the tonsils that may simulate Vincent's angina or severe diphtheric destruction of the tonsils. The petechiae are scattered throughout the body. There usually is enlargement of the lymphatic glands and enlargement of the spleen. The thrombocytes usually are not diminished in number and are sometimes even increased. The bleeding time is not interfered with and the retractability of the blood clot is firm. The chronic type of lymphatic leukemia is so different in its symptomatology from thrombocytopenia that the differential diagnosis does not enter into consideration.

The acute aleukemic and subleukemic types of myeloblastic leukemia do not, as a rule, show the same hemorrhagic manifestations as does thrombocytopenia. The spleen usually is enlarged in these cases. The thrombocytes are only moderately reduced in number in proportion to the anemia. The bleeding time is normal and the retractability of the blood clot is firm. The myeloblasts and the myelocytes give a positive oxydase reaction. It is also important to remember that either form of acute leukemia may set in gradually and that the active symptoms may be preceded by pain in the gums which causes the patient to consult a dentist who extracts one or more teeth. The severe systemic symptoms that follow are then attributed to the extraction, and the dentist often is innocently sued for malpractice.

APLASTIC ANEMIA

Another disease with which the symptoms of thrombocytopenia has much in common is aplastic anemia. Aplastic anemia is a term introduced by Ehrlich, who observed a case of pernicious anemia in which the normoblasts disappeared from the blood and the color index became low. He predicted that there would be rapid fatal termination of this case, as the blood showed that the regenerative power of the bone marrow had disappeared. He also predicted that the bone marrow of the long bones would be yellow instead of red because of the absence of regenerative power of the bone marrow. The necropsy proved this assumption to be correct. Ehrlich therefore reserved the use of the term aplastic anemia to the terminal stage of pernicious anemia, or to those cases of pernicious anemia that run a fulminating fatal course without showing any regenerative power on the part of the bone marrow. For a time both clinicians and hematologists accepted Ehrlich's view with regard to aplastic anemia. As cases of anemia with absence of regenerative power of the bone marrow began to

accumulate without any evidence of pernicious anemia, clinicians and hematologists considered aplastic anemia as an independent clinical picture. According to Eppinger, the disease sets in acutely in the midst of perfect health, with hemorrhages from the internal organs, often from the uterus. Sometimes there is severe epistaxis. At times the continuous oozing of blood rapidly leads to severe secondary anemia, and quite often there is a reduction in leukocytes. Not infrequently the thrombocytes also are diminished in number, the disease then resembling malignant thrombocytopenia. It is sometimes difficult to differentiate the disease from thrombocytopenia, as in some cases petechiae are also present. In contradistinction to pernicious anemia, the subicteric tinge of the skin and of the sclerae is absent in aplastic anemia. This fact was noted by Turck and confirmed by Eppinger. These two authors also pointed out that urobilinuria—an important factor in pernicious anemia—is not so pronounced in aplastic anemia. Both phenomena, that is, the absence of subicteric tinge of the skin and of the sclerae together with the absence of urobilinuria, indicate that the process of blood destruction is not the same in pernicious as in aplastic anemia. Further proof that in aplastic anemia the destruction of the blood is of different nature from that in pernicious anemia is brought by Eppinger in a case studied both clinically and pathologically. Clinically, he found normal acidity of the stomach, no urobilin in the urine, no increase of bilirubin in the blood, a normal output of iron in the stool—it differed in every respect from the destruction of pernicious anemia. The postmortem findings showed sulphur yellow bone marrow of the long bones, but no hemosiderosis in the liver or in the spleen. Wallgren's [22] experience, based on clinical and pathologic studies, confirms Eppinger's findings. Another important differentiating point between pernicious and aplastic anemia is the fact that in the latter there are never any changes of subacute combined degeneration of the spinal cord, as is so often the case in the former. It must therefore be accepted that aplastic anemia is a clinical entity that may occur in the course of pernicious anemia or secondary to severe hemorrhages or often may appear without any known cause, as does essential thrombocytopenia. The differential diagnosis between aplastic disease and essential thrombocytopenia would, under such conditions, be impossible.

Severe sepsis, especially of streptococcic or staphylococcic origin, may sometimes run a course simulating malignant thrombocytopenia. The blood picture in sepsis, however, is that the polymorphonuclear cells are markedly increased even if there is a leukopenia. The repeated chills as well as the marked delirium also are factors aiding in the differential

22. Wallgren, Ivor: Arb. a. d. Path. Inst. d. Univ. Helsingfors, 1925, pp. 275, 366.

diagnosis. Severe hemorrhages such as are encountered in malignant thrombocytopenia are rare in sepsis.

AGRANULOCYTIC ANGINA

In 1922 Schultz and Verse [23] and Friedeman [24] and, soon after, Leon [25] described a clinical picture under the name angina agranulocytotica, occurring usually in middle aged women, which was attributed to an intoxication or an infection. It has an acute onset, with high temperature, necrosis of the mucous membrane of the mouth and pharynx, marked leukopenia and absence of neutrophilic leukocytes. This disease terminates fatally. At the necropsy the liver and the spleen are normal but the bone marrow shows marked pathologic changes. A number of cases have since been reported. Skiles [26] described a case fully under the term agranulocytic angina, and Piette [27] studied the histopathology of a similar case. The last named author, in contradistinction to the others, found that the structure of the spleen was markedly changed and that there was enormous development of the sinus endothelial system at the expense of the lymphatic tissue. The endothelial cells comprised about 90 per cent. of the entire splenic substance. The sinuses appeared empty in most places but in some places they contained mycotic emboli, a few lymphocytes and an occasional plasma cell. Not a single polymorphonuclear cell was to be seen either in the spleen or in any other organ. In the capsule of the spleen and of the liver numerous bacilli were present. In some of the capillaries, bacterial emboli were encountered, a condition common in practically all forms of sepsis. The most pronounced changes were in the kidney. Innumerable bacterial emboli were distributed over the cortex and were particularly abundant in the region of the vas afferens and in the glomeruli, but without any inflammatory reaction. The suprarenal glands also contained numerous emboli in the capsules of the cortical substances without any evidence of inflammation. The remaining organs showed areas of superficial necrosis. In view of the fact that numerous bacilli were found in the various organs, Piette asserts that the disease is of bacillary origin and is possibly due to *Bacillus pyocyaneus*. Whether the term agranulocytic angina should be used for a distinct disease or merely as the expression of severe sepsis is still unsettled. It resembles malignant thrombocytopenia in its mouth lesions, in the marked secon-

23. Schultz and Verse: Deutsch. med. Wchnschr., 1922, No. 44.
24. Friedeman, U.: Med. Klin., No. 41, 1922-1923.
25. Leon, A.: Deutsch. Arch. f. klin. Med. **143**:118 (Aug.) 1923.
26. Skiles, J. H.: Agranulocytic Angina, J. A. M. A. **84**:364 (Jan. 31) 1925.
27. Piette, E. C.: Histopathology of Agranulocytic Angina, J. A. M. A. **84**:1415 (May 9) 1925.

dary anemia, and especially in the disappearance of polymorphonuclears and eosinophils from the blood. It differs from essential thrombocytopenia by the fact that there is but slight if any diminution in thrombocytes, by the absence of petechiae or hemorrhages from internal organs and by the normal bleeding time and the normal retractability of the blood clot.

PATHOGENESIS

The question is still unsettled as to the true cause of the sudden reduction of thrombocytes in thrombocytopenia.

Kaznelson believes that the thrombolytic action of the spleen is the primary factor; he bases his assertion on the fact that in thrombocytopenia the removal of the spleen has a curative effect and that in some cases the spleen is enlarged. Kaznelson also asserts that in these cases a marked increase in destroyed blood platelets is found in the sinuses of the removed spleen. While it is true that splenectomy exercises a beneficial effect on the symptoms of thrombocytopenia, it by no means brings about a cure. The bleeding as well as the retractile property of the blood clot returns to normal; the number of thrombocytes increases only temporarily and within a few weeks again reaches the point it was before the operation; sometimes it does this a few days after the operation. The disappearance of the hemorrhages after splenectomy would indicate, according to Brill and Rosenthal, that an agency is present in the normal spleen which has some controlling mechanism on the capillary activity through the medium of the blood platelets.

The fact that the bleeding ceases even in the presence of a diminished number of thrombocytes after splenectomy in this disease would also show, according to these authors, that normally the spleen influences the two important properties of the thrombocytes, namely, the one that produces blood retraction and the agglutinative power to form thrombi. These properties may be restored immediately after the removal of the spleen or by a later steady development. The process involved is therefore not due to the spleen exercising an inhibitory or destructive property on the megakaryocyte but to the harmful influence of the reticuloendothelial system on the thrombocyte.

Another proof that the spleen is not the primary cause of thrombocytopenia is the fact that most observers fail to find an increase of destroyed thrombocytes in the sinuses of the removed spleen. In reality there is a decrease of these elements. Klemperer, in a personal communication, said that he had studied a number of such spleens in the Postgraduate Pathological Laboratory, and found this organ absolutely normal. The cases quoted by Kaznelson and Keisman, in which the spleen was enlarged with an increase in destroyed thrombocytes in the

sinuses, in all probability belong to symptomatic purpura secondary to splenomegalic disease.

Frank is of the opinion that the primary factor is the failure of the megakaryocyte to produce a sufficient number of thrombocytes. Most authors, including Eppinger and Christian,[28] adhere to that theory.

It seems reasonable to assume that in essential thrombocytopenia due to some unknown toxin of infectious, chemical or metabolic nature, the megakaryocytes become affected and thereby produce an insufficient number of thrombocytes of an inferior quality. The normal number of thrombocytes is undoubtedly in excess and serves the purpose of reserve. The severity of disease of any organ depends on the rapidity with which the reserve is used up. In the case of essential thrombocytopenia the reserve is quickly exhausted because of diminished production by the megakaryocyte and the destructive action of the spleen. It cannot be definitely stated whether the inhibitory factor lies in the spleen alone or in the entire reticulo-endothelial system. In view of the fact that shortly after the removal of the spleen the thrombocytes are again reduced and sometimes some of the symptoms recur is a proof, as stated by Brill and Rosenthal, that the remaining reticulo-endothelial system may also exert a detrimental effect on the megakaryocyte. It is of the utmost clinical and therapeutic importance in every disease to trace the lesion to its original source. In cardiac disease, for instance, the diagnosis would be incomplete unless one localized the affection either in valve or heart muscle. Diseases affecting the hematopoietic system must likewise be traced to the original source, although the symptoms may be similar irrespective of where the primary affection lies. If the erythroblastic elements are primarily affected, polycythemia or pernicious anemia results; the affection of the mother cell of the leukocyte is responsible for leukemia, and the affection of the mother cell of the thrombocyte causes thrombocytopenia and, in rare cases, an increase in thrombocytes. Although one of the three named mother cells may be first affected, it is self understood that the others become coaffected in the course of time, and that eventually the entire hematopoietic system becomes involved. This explains why in pernicious anemia there are eventually associated changes in leukocytes and thrombocytes. Leukemia in turn affects the erythrocytes and thrombocytes; thrombocytopenia in the benign form affects mainly the blood platelets, but in the severer form the erythrocytes and leukocytes are likewise affected, bringing about a marked reduction in polymorphonuclear leukocytes, absolute absence of eosinophils and a great reduction in red blood cells.

A primary disease of the hemolymph glands or of the liver, in the course of time, brings about pathologic changes in the hematopoietic

28. Christian, Henry: Oxford Medicine **2**:777-797.

part of the bone marrow. Lymphogranuloma, for instance, causes a reduction in red blood cells as well as marked leukocytosis and mononucleosis. Splenomegalic anemia eventually affects the hematopoietic quality of the bone marrow, bringing about mononucleosis and severe anemia and mucous membrane hemorrhages.

SYMPTOMATIC PURPURA

In contradistinction to primary essential thrombocytopenia, other forms of hemorrhagic diathesis, with reduction in blood platelets or without such reduction, should be termed secondary purpura or symptomatic purpura. The two most important diseases of this group are those described by Schoenlein and by Henoch. These two diseases are also known as hemorrhagic capillary toxicosis or anaphylactoid purpura. The main characteristics of these diseases are the pathologic changes in the capillaries.

SCHOENLEIN'S PURPURA

The following clinical manifestations are present in Schoenlein's purpura: edema and swelling of the joints; purpuric spots of varying sizes, at first bright red in color and later dark, appearing mostly on the lower extremities. The purpuric spots show a marked tendency to appear in groups and to recur. The duration of the disease is from one to six weeks. After the disappearance of the purpura, if the patient is out of bed there may be a rapid recurrence. Schoenlein states that the petechiae are principally confined to the areas around the ankles, and that they are never larger than a pea. It is well known, however, that the petechiae may occur over other parts of the body, especially over the extensor and the flexor surfaces of the extremities, and that they may at times be as large as an urticarial wheel or take the form of circumscribed erythema. The hemorrhagic areas are surrounded by a red zone and sometimes by hemorrhagic nodules resembling erythema nodosum. The disease is also named rheumatic peliosis. Ottenberg[29] rightfully states that this term should not be used because it gives the erroneous impression that the disease is allied to acute articular rheumatism. In reality, as Ottenberg pointed out, it has nothing in common with polyarticular rheumatism. It is never preceded by tonsillitis, never favorably influenced by salicylates; it is never complicated by endocarditis nor do the joint affections ever become chronic. Unlike polyarticular rheumatism, it is not hereditary nor familial. The capillary stasis test is usually positive in Schoenlein's disease and negative in polyarticular rheumatism. The positive test is not due to the diminution of the platelets because they are normal in number in this disease, but to the diseased state of the capillaries. There is never leukocytosis in

29. Ottenberg, R.: M. Clin. N. A. **2**:289 (July) 1918.

Schoenlein's purpura, while in polyarticular rheumatism, as demonstrated by the Rockefeller Institute workers in New York, there is a leukocytosis with a polynucleosis. The temperature is only moderately elevated in Schoenlein's disease, while in polyarticular rheumatism it is high. The pain in the joints in Schoenlein's disease is never as severe as in acute articular rheumatism.

HENOCH'S PURPURA

Henoch's purpura is a more severe affection than Schoenlein's purpura. This disease frequently affects the mucous membrane of the internal organs, especially of the gastro-intestinal tract. The symptoms may begin with severe gastro-intestinal pain preceding the appearance of cutaneous hemorrhages. Sometimes the gastro-intestinal symptoms alone may be present, simulating either appendicitis or some other gastro-intestinal disease. This makes the differential diagnosis difficult. The gastro-intestinal symptoms are due to hemorrhagic exudation into the intestinal wall. If this exudate affects the appendix, it causes marked distention of the organ with accompanying symptoms, and even at operation the distended appendix simulates an acute suppurative appendix. If an exudation into the intestinal wall occurs, especially one into the colon, bloody diarrheal stools or rectal tenesmus are present. The hemorrhages sometimes occur into the kidney, causing hemorrhagic nephritis and, in severe cases, anuria and retention of nitrogenous products in the blood. Occasionally the hemorrhage into the kidney may cause clotting of blood in the ureter, with symptoms of renal colic simulating renal calculus. Hemorrhages into the brain may occur, causing edema of the brain and convulsions. Hemorrhagic effusions into the joints are rare. Henoch's purpura may be familial and is subject to recurrence. The thrombocytes are diminished only in proportion to the anemia. The bleeding and coagulation time are not interfered with in either of these diseases.

Christian and others class the Schoenlein and Henoch's purpura with the various forms of erythema accompanied by abdominal symptoms first described by Osler.[30] While it is true that these erythemas are frequently associated with abdominal symptoms, actual purpura and hemorrhages from the mucous membranes or intestines or other organs are usually absent in the erythema type of cases.

The etiology of the Schoenlein and Henoch's purpura is not definitely known though there is no doubt that anaphylaxis plays a rôle.

Purpuric hemorrhages under the skin, and even under the mucous membrane, may be secondary to any disease of the hematopoietic system, but in most instances they are seen together with leukemias and spleno-

30. Osler: Am. J. M. Sc. **127**:1, 1904.

megalic anemias. In the lymphatic type of leukemia the hemorrhages occur chiefly from the gums, the mucous membrane of the mouth, the pharynx and, less frequently, from the nose. In splenomegalic anemia, especially in the so-called Banti symptom complex, hemorrhages set in late in the disease, and most frequently from the gastro-intestinal tract, especially the stomach. In Gaucher's disease, hemorrhages are not common, but when they are observed they usually occur late in the disease. In these cases neither the coagulation nor the bleeding time is interfered with. The number of blood platelets is reduced, but is never out of proportion to the degree of anemia. In splenomegalic hemorrhages the spleen aids in the destruction of the thrombocytes because in these cases the sinuses of the removed spleen show large numbers of destroyed thrombocytes. Purpura may be secondary to severe infections, such as streptococcic and staphylococcic bacteremia, scarlet fever, measles, smallpox or typhus exanthematicus. It may be found in the aged and weak. It may occur in the terminal stages of secondary contracted kidney. Cutaneous hemorrhages are seen in the course of affections of the liver without icterus but they are more frequent when icterus accompanies the liver disease.

ENDOTHELIOSIS HEMORRHAGICA (MORBUS LITTEN)

Endotheliosis hemorrhagica was first observed by Litten in association with bacterial endocarditis (Libman). It is characterized by the presence of macrophages in the blood which possess a phagocytic property. In addition to this, there is a decrease in the number of neutrophils and a relative increase in lymphocytes. It seems that in this affection the defense against the streptococcus viridans is characterized by the absence of a myelocytic element which normally causes an increase in the neutrophilic leukocytes. As the studies of Kuczinski and Wolf show, the defense is the work of the endothelial elements of the endothelium, the spleen and the kidney. These findings are confirmed by Libman, Rosenthal and many others. Libman is inclined to ascribe great importance to the macrophages in the process of immunity. In bacterial endocarditis the petechiae are characteristic of hemorrhagic diathesis; hemorrhages from the mucous membrane of internal organs are absent. The capillary stasis test usually is positive, but bleeding and coagulation time are normal and the number of thrombocytes is moderately reduced.

TREATMENT OF ESSENTIAL THROMBOPENIA

As the specific cause is unknown, it is evident that the treatment must be symptomatic. Blood transfusion of either direct or citrated blood has been advocated. The beneficial effect of blood transfusions in this

disease is not as startling as the results following hemorrhages, or as is sometimes the case in pernicious anemia or anemia due to leukemia. It should be tried, but should not be repeated if the hemorrhages are aggravated as sometimes happens. Cacodylate of soda, from 1 to 3 grain (0.06 to 0.19 gm.) doses intramuscularly every day may be employed. For the symptoms of collapse hypodermoclysis with 250 c.c. of Ringer's solution is of great value. The calcium contained in the Ringer's solution also has a beneficial hemostatic effect. In the protracted and recurrent type of cases, the removal of the spleen as proposed by Kaznelson should be resorted to. The thrombocytes usually increase in number soon after the operation.

In a case reported by Charlotte Ehrenberg the blood platelets, which were 10,000 before the operation, rose to 87,000 two and a half hours after the operation, and the bleeding stopped at once. In some cases a few days after the operation the thrombocytes may rise as high as 500,000, which is more than normal. There is a case on record in which the blood platelets increased to 1,500,000 after the operation. After the initial increase the blood platelets again began to decrease in number, coming down as low as 50,000 or less. In spite of this the bleeding does not recur, although some of the phenomenon, like the Rumpell-Leede, may be present again. The removal of the spleen is not as a rule difficult and does not cause severe operative or postoperative bleeding. In a number of cases, however, suppuration in the left subdiaphragmatic space occurs after operation, and in many cases left sided pneumonia occurs. In the severe aplastic type of anemia the removal of the spleen is useless. Another treatment to be resorted to is the roentgen-ray treatment of the bone marrow and spleen and, sometimes, during the critical period, epinephrin injections and foreign protein may be used. Severe bleeding is also sometimes controlled by the intravenous use of from 10 to 30 c.c. of 10 per cent. sodium chlorid solution or 20 c.c. of 10 per cent. calcium chlorid solution; the latter should be given with great care directly into the vein, avoiding tissue infiltration because necrosis results if a drop of calcium chlorid solution escapes into the tissues. To avoid the necrosis that may follow calcium chlorid injection when leakage occurs into the tissue, Seelig [31] advises the use of a 1 per cent. calcium solution, the total amount being the same.

Treatment of Schoenlein and Henoch's purpura is chiefly symptomatic. Schoenlein's type usually runs a mild course and recovery ensues without any treatment. If the pains in the joints are troublesome, wet dressings and codein combined with calcium lactate internally

31. Seelig, M. G.: Localized Gangrene Following the Hypodermic Administration of Calcium Chlorid, J. A. M. A. **84**:1413 (May 9) 1925.

are beneficial. In Henoch's purpura, some authors advise large doses of animal charcoal, from 20 to 30 gm. daily, for several days. In the severe cases, intravenous injections of from 0.5 to 1 liter of Ringer's solution are beneficial. The calcium contained in them is supposed to influence the capillaries favorably. Calcium lactate in one-half tablespoonful doses is likewise advised.[32]

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32. It may be of clinical interest to record a personal communication from Dr. A. L. Amsterdam describing a patient who developed purpuric spots each time she took phenolphthalein.

THE VALUE OF A STARCH-IODIN REACTION AS A TEST OF PANCREATIC FUNCTION *

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In 1924 Bassler devised a test for determining pancreatic function. With a view to establishing the practicability of this test and its adaptability for clinical purposes, this work was begun. We soon observed such marked variations in the results obtained by varying one of the reagents that we were led to undertake a detailed investigation of the test itself and of the various factors entering into it.

The Bassler test is presumably a test for pancreatic amylase and is designed to determine quantitatively that enzyme alone. In order to be of value as an index of pancreatic function this test presupposes the nondissociation of pancreatic enzymes; that is, if one enzyme is decreased the others are assumed to be proportionately diminished. This point is still far from settled. Against this nondissociation view may be cited the conclusions of Pawlow,[1] that the kind of food eaten calls forth an increase in the secretion of the enzyme specific for that particular type of food. The work of Einhorn,[2] the application of his method in the study of normal subjects by Friedenwald and Sindler,[3] the recent study by two of us [4] on cholecystitis, as well as the complicated but probably more accurate methods (with regard to the basic principles of the chemistry of enzyme action) of McClure, Jones, Wetmore and Reynolds [5] all tend to refute this view. The latter observers found, like Pawlow, that there was a relationship between the kind of food ingested and the enzyme concentrations of duodenal contents. They also found that in a study of a group of cases that gave readings between normal and subnormal a dissociation of the concentration of the various types

* From the gastro-intestinal clinic of the hospital of the Graduate School of Medicine of the University of Pennsylvania.

1. Pawlow, I. P.: The Work of the Digestive Glands, London.

2. Einhorn, Max: The Duodenal Tube, Philadelphia, W. B. Saunders Company, 1921.

3. Friedenwald, J., and Sindler, J.: Fractional Analysis of the Duodenal Contents in Normal Individuals, J. A. M. A. **177**:1469 (Nov. 5) 1921.

4. Piersol, G. M., and Bockus, H. L.: Pancreatic Enzymes in Cholecystitis, Arch. Int. Med. **35**:204 (Feb.) 1925.

5. McClure, C. W.; Jones, C. M.; Wetmore, A. S., and Reynolds, L.: Studies in Pancreatic Function; Enzymic Concentrations of Duodenal Contents in Health and Disease, Am. J. M. Sc. **167**:649-664 (May) 1924.

of enzymes occurred. This dissociation was characterized by a normal concentration of one or two enzymes while the remaining ones were much diminished below the minimum normal. Meyner [6] also found that he was unable to recognize any definite relationship among the individual enzymes, and Spencer [7] felt convinced that he could demonstrate a greater stimulation of a specific enzyme by a specific food.

The work of other investigators, on the other hand, tends to substantiate Bassler's view as to the nondissociation of pancreatic enzymes. Grote [8] states that the general variation of the three ferments is uniform in their relationship. Wohlgemuth,[9] in repeating Pawlow's experiments on pancreatic juice obtained through a traumatic pancreatic fistula in a man, was not able to demonstrate any relationship between the type of foodstuff fed and the degree of activity of the enzyme specific for that kind of food.

The technic of the Bassler test as we employed it was as follows: A duodenal tube was passed into the duodenum on an empty stomach, preferably in the morning. When the tip of the tube was ascertained to be in the duodenum, we used the method described by Lyon;[10] 100 c.c. of 5 per cent. Witte peptone solution was injected through the tube as an activator to the pancreas. The tube was clamped off for five minutes; then the duodenal contents were aspirated. The first fraction aspirated was used for the test, because Bassler [11] states that this contains the largest portion of pancreatic juice and represents the sudden liberation of the stored up secretion of the pancreas.

The following reagents were employed:

Solution A, prepared as follows: In a beaker 2 gm. of corn-starch (Duryea) were placed; to this were added 100 c.c. of cold distilled water. This was mixed thoroughly and then heated. Under constant stirring the mixture was brought to boiling and then cooled.[12]

Solution B, a 1 per cent. sodium chlorid solution. The standard buffer solution mentioned in the original description of the test was omitted as it is now regarded as nonessential.

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6. Meyner, E.: Study on Pancreatic Function, Med. Klin. **16**:678 (June 27) 1920.

7. Spencer, G. F.: J. Lab. & Clin. Med. **9**:261-267 (July) 1920; ibid. **9**:261 (Jan.) 1924.

8. Grote, L. R.: Diagnosis of Pancreas Function from Duodenal Contents, Zentralbl. f. inn. Med. **43**:777-792 (Dec.) 1922.

9. Wohlgemuth, J.: Berl. klin. Wchnschr. **44**:47, 1907.

10. Lyon, B. B. V.: Nonsurgical Drainage of the Gallbladder, Lea & Febiger, 1923, p. 313.

11. Bassler, A.: A Quantitative Test of Digestive Pancreatic Activity Easily Applied Clinically, Arch. Int. Med. **35**:162 (Feb.) 1925.

12. Ferris, H. C.; Smith, E. E., and Graves, E. V.: J. Am. Dent. A., January, 1923

The standard Bassler reagent was then prepared by combining solutions A and B, as follows: To Solution A, when cool, 25 c.c. of Solution B was added and enough distilled water to bring the volume of the mixture up to exactly 400 c.c. This standard reagent was freshly prepared each day.

In addition to these solutions, at first a tenth normal iodin solution, kept in a bottle with a dropper cork, was prepared. After the first series of tests, this solution was changed to a twenty-fifth normal iodin solution, a strength which has been found more satisfactory and to which we have adhered in all our subsequent work.

The actual procedure employed in carrying out the complete test is a modification of the method devised by Smith [12] in testing for ptyalin.

Ten tubes of equal size were placed in a rack and numbered from 10 to 1. Into each of the ten tubes there was accurately pipetted, with a 1 c.c. pipet graduated in hundredths, definite quantities of duodenal return, water and the standard reagent. The quantities in cubic centi-

TABLE 1.—*Quantities of Duodenal Return, Water and Standard Reagent*

	Duodenal Return, C.c.	Water, C.c.	Bassler's Reagent, C.c.	Result in Bassler Units, per 100 C.c.
Tube 10	0.1	0.9	4	20
Tube 9	0.11	0.89	4	18
Tube 8	0.125	0.875	4	16
Tube 7	0.14	0.86	4	14
Tube 6	0.17	0.83	4	12
Tube 5	0.2	0.80	4	10
Tube 4	0.25	0.75	4	8
Tube 3	0.33	0.67	4	6
Tube 2	0.5	0.5	4	2
Tube 1	1.0	0.0	4	2

meters of these materials that are added to each tube are shown in Table 1.

When prepared and shaken, the rack was put in an incubator at 38 C. for forty minutes. The rack of tubes was then taken out; the contents of each tube was shaken, and one drop of tenth normal solution of iodin was added to each tube. Twenty minutes later the estimation of the amount of pancreatic amylase in terms of Bassler units was made according to readings obtained from the furthest tube to the left that was achromic. When there was no achromic tube, but instead one side of the rack was pink and the other side green, the reading was taken between the adjacent pink and green tubes. The result in Bassler units was obtained by multiplying the number of the achromic tube by 2. The details of these calculations may be found in Bassler's original article.[11] The normal reading is supposed to lie between 8 and 14 units, the unit being defined as the quantity of amylase that will digest 1 gm. of starch under the conditions outlined.

In the first series of cases on which we tried the test as described above, we were struck by the consistently low readings obtained. These low readings occurred in spite of the fact that in the series there were several patients in whom there was no reason to suspect any pancreatic deficiency (Table 2, Series A). In this series there were fourteen cases, of which only two gave readings of 4 units each, the highest reading obtained in this group.

In accordance with a subsequent correction in the technic offered by the originator of this method, the iodin used for indicator was changed from tenth normal to twenty-fifth normal. The test was then repeated with this modification, the other reagents being unchanged. This group consisted of twelve cases, some of which had been used in the first series (Table 2, Series B). We now found that the results obtained

TABLE 2.—*Variabilities of the Reaction with Different Amounts of Iodin Solution*

Series A			Series B		
Case	Pancreas at Operation	Results in Bassler Units with Tenth Normal Iodin, 3 Drops	Results in Bassler Units with Twenty-fifth Normal Iodin, 1 Drop	Pancreas at Operation	Case
1	Normal	2	20	Normal	1
2	.....	3	20	.....	2
3	.....	3	20	.....	3
4	Shotlike areas in head	4	20	Normal	15
5	.....	3	20	.....	16
6	.....	3	20	.....	17
7	.....	4	20	Slight hardening of head	18
8	.....	2	20	Normal	19
9	.....	0	14	.....	20
10	Chronic interstitial pancreatitis; biopsy	0	14	.....	21
11	.....	2	20	.....	22
12	.....	3	20	.....	23
13	.....	0			
14	.....	3			

were all consistently too high. There were nine cases with readings of 20 units each, and the lowest obtained in the series was a reading of 14 units.

In doing the test we had noted what a marked variation in the reaction could be produced by varying the amount of iodin added. This led us to seek an explanation of this phenomenon, which appeared a serious objection to the test. Dr. Wilson, professor of physiologic chemistry in the University of Pennsylvania, on being consulted, suggested the possibility that some of the substances entering into the reaction might have the power of taking up iodin. He looked on the peptone or unsaturated fatty acids in the bile or duodenal juice as substances likely to possess such power. On his suggestion, therefore, we undertook an investigation of each substance entering into the reaction with a view to determining their iodin absorption power.

IODIN ABSORPTION BY PEPTONE

Four series of ten tubes each were taken. Peptone solution of varying strength was used in each series, namely, 0.25 per cent., 0.5 per cent., 1 per cent. and 2 per cent. The strength and quantities of the other reagents were the same as used in the Bassler test. To each tube there was then added 1 drop of twenty-fifth normal iodin solution and the tubes were then allowed to stand at room temperature for twenty minutes. At the end of this time, 4 c.c. of Bassler starch solution was added to each tube with the following results:

0.25 Per Cent. Series: Tubes 1 and 2, very slight starch-iodin reaction; Tubes 3 to 10, inclusive, gave a definite starch-iodin reaction.

0.5 Per Cent. Series: Tubes 1, 2 and 3, achromic; Tubes 4 to 10, inclusive, definite starch reaction.

1.0 Per Cent. Series: Tubes 1 to 5, inclusive, achromic; Tubes 6 and 7, very slight starch-iodin reaction; Tubes 8, 9 and 10, definite starch-iodin reaction.

2.0 Per Cent. Series: All tubes achromic.

These observations suggested that the amount of iodin absorbed varied directly with the strength of the peptone solution present.

IODIN ABSORPTION BY DUODENAL FLUID

Duodenal tubes were passed on three fasting patients. When the tip was ascertained to be in the duodenum, the tube was connected to a drainage bottle and the duodenal residues collected by siphonage.

Three sets of ten tubes each were taken. To one set, 0.5 c.c. quantities of duodenal residuum from Patient 1 were placed in each tube. The second set contained like amounts of duodenal residuum from Patient 2, and in the tubes of the third set 0.5 c.c. of duodenal residuum from Patient 3 was placed. Twenty-fifth normal iodin solution was then added to the tubes of each series, starting with 1 drop in Tube 1, and increasing the amount added to each successive tube by 1 drop, until 10 drops were added to Tube 10. The tubes were then shaken and allowed to stand at room temperature for twenty minutes. At the end of this time 4 c.c. of Bassler starch solution was added to each tube. The results were as follows:

Patient 1: Tubes 1, 2 and 3, achromic; Tube 4, slight starch-iodin reaction; Tubes 5 to 10, inclusive, definite starch-iodin reaction.

Patient 2: Tubes 1 to 5, inclusive, achromic; Tubes 6 to 10, inclusive, definite starch-iodin reaction.

Patient 3: Tubes 1 to 9, inclusive, achromic; Tube 10, definite starch-iodin reaction.

Thus it appears that the duodenal fluid itself introduces a second important variable with regard to iodin absorption. Incubation of the juice before adding the iodin had no effect on this absorption power. The duodenal fluid was also found capable of splitting the starch-iodin combination, with the removal of the color from a starch-iodin solution. Similar reactions were carried out with duodenal contents after stimu-

lation with peptone. It was found that this fluid took up more iodin than either the peptone solution or the fasting duodenal juice alone.

This is shown by comparing the figures in Table 3. The first two columns represent readings obtained by carrying out the Bassler test on fasting duodenal residuum. It will be noted that a marked difference in the readings is brought about by increasing the addition of iodin by

TABLE 3.—*Variations in Results, Expressed in Bassler Units, of Regular Bassler Test when Carried Out with Varying Quantities of Iodin on Duodenal Residuum Before and After Stimulation with Peptone Solution*

	Fasting Duodenal Residuum		Duodenal Residuum After Peptone	
Case	Twenty-Fifth Normal Iodin, 1 Drop	Twenty-Fifth Normal Iodin, 2 Drops	Twenty-Fifth Normal Iodin, 1 Drop	Twenty-Fifth Normal Iodin, 2 Drops
1	8	2	20	14
2	4	2	20	14
3	12	2	20	18
4	6	2	20	20
5	5	4	20	20
6	20	6	20	20

TABLE 4.—*Two Types of Technic*

Technic of Bassler Test	Modified Technic
1. Pass duodenal tube	1. Pass duodenal tube
2. Stimulate pancreas with 5 per cent. Witte peptone solution	2. Stimulate pancreas with 5 per cent. Witte peptone solution
3. Aspirate duodenal contents after 5 minutes	3. Aspirate duodenal contents after 5 minutes
4. Prepare 10 tubes as for test	4. Prepare 10 tubes as for test
5. Incubate	5. Incubate
6. Add 1 drop of twenty-fifth normal iodin to each tube	6. Determine iodin absorption factor: a. Use 10 tubes with 0.5 c.c. of duodenal return in each tube b. Put 0.05 c.c. of twenty-fifth normal iodin in Tube 1, increasing by 0.05 c.c. in each succeeding tube to 0.5 c.c. in Tube 10 c. Shake; stand at room temperature 30 minutes d. Add 4 c.c. of Bassler starch solution to each tube e. Read achromic tube containing largest amount of iodin f. Calculate amount of iodin absorption for each tube in test g. Add calculated amount of twenty-fifth normal iodin to each tube plus 0.05 c.c. in excess
7. Read in 20 minutes	7. Read in 20 minutes

a single drop. In the other two columns, which represent the results of the Bassler test done on duodenal residuum after peptone stimulation, the change occasioned by an additional drop of iodin is very slight. The explanation of these variations lies in the fact that in fasting duodenal residuum, we have only one substance or group of substances which is capable of taking up iodin; while in the peptone stimulated duodenal residuum an additional substance capable of iodin absorption also is

present. In other words, the addition of a second drop of iodin to the duodenal residuum alone furnishes enough excess iodin to bring out a starch-iodin reaction in several additional tubes; whereas, in the tubes containing both the peptone and the duodenal juice, there was present another substance capable of taking up the additional iodin so rapidly that no change or only a slight change in the readings occurred.

The foregoing observations furnish an explanation for the wide variation in the results obtained by varying the quantity of iodin employed in the test. In other words, there are substances present in this test which are capable of taking up such large quantities of iodin, as compared to the amount used in the test as originally proposed, that it is unnecessary to have any starch digestant present in order to obtain achromic tubes. Hence, readings suggestive of the presence of amylase may be obtained even if there is no pancreatic juice at all in the material tested.

In order to overcome this apparently serious objection to the test and to increase its reliability by accounting for the important variables that were obviously present, it seemed necessary to determine the iodin absorptive power of the duodenal return after peptone stimulation and to account for it in some way in the final step of the reaction. This was accomplished as follows: All the steps in technic as originally described were carried out until the final one. At this point a method was devised for determining what may be called the iodin absorption factor for the duodenal return.

A series of ten tubes numbered from 1 to 10 were taken. In each tube was placed 0.5 c.c. of duodenal return. To Tube 1 there was added 1 drop of twenty-fifth normal iodin solution. The addition of iodin solution to each succeeding tube was increased by 1 drop; thus, 10 drops were added to Tube 10. The contents of each tube were mixed thoroughly and allowed to stand at room temperature for thirty minutes. This provided adequate time for saturation of the duodenal return with iodin. To each tube 4 c.c. of Bassler starch solution was then added and the tube with the largest amount of iodin which failed to show any starch iodin reaction was then determined. The number of this tube was multiplied by 2. The result showed the number of drops of twenty-fifth normal iodin which 1 c.c. of that particular duodenal return was capable of absorbing. From this figure the iodin absorptive power of the quantity of duodenal return in each tube of the Bassler test was calculated, e. g., for Tube 10, one tenth of the amount; for Tube 9, eleven-hundredths, and so on. The final step in the test, when the iodin was to be added, being reached, the calculated amount of iodin necessary to take care of the iodin absorption factor as previously determined plus one drop in excess of this amount was added to each tube. The

determined amount was to allow for the absorption by the duodenal return, and the additional drop to react with any starch or products of starch digestion which still remained.

It soon became apparent that if the iodin was to be added accurately quantitatively, the drop method was not sufficiently exact. Therefore, we changed to the pipet for determining the iodin absorption factor. An accurately calibrated pipet was employed and for each drop used in the method outlined, 0.05 c.c. was substituted.

Table 4 shows a comparison between the original Bassler test and the same test as modified by the estimation of the iodin absorption factor.

Table 5 shows the difference in the results obtained on the same patient when the regular Bassler test was employed and when the test was modified by determining and allowing for the iodin absorption factor. In the latter modification it will be noted that even after repeated estimations the results are encouragingly uniform.

TABLE 5.—*Comparison of Bassler Test with Its Modification*

		Modified Technic											
Case	Regular Bassler Test Results in Bassler Units	Bassler Units	Iodin Absorption Factor, C.c.	Bassler Units	Iodin Absorption Factor, C.c.	Bassler Units	Iodin Absorption Factor, C.c.	Bassler Units	Iodin Absorption Factor, C.c.	Bassler Units	Iodin Absorption Factor, C.c.	Bassler Units	Iodin Absorption Factor, C.c.
1	20	4	0.3	5	0.5	4	0.3	4	0.5	3	0.4	3	0.4
2	10	0	0.5	0	1.0	0	0.3	0	0.4	..	...	..	...
3	12	4	1.0	3	0.6	..	...	..	...	..	...	..	...
4	20	20	0.9	18	0.7	18	0.7	18	0.8	..	...	..	...
5	12	3	0.6	5	0.4	..	...	..	...	..	...	..	...
6	20	3	0.3	4	0.3	4	0.2	..	...	..	...	..	...
7	20	10	0.5	10	0.3	7	0.5	8	0.5	..	...	..	...
8	20	6	0.5	5	0.4	7	0.4	..	...	..	...	..	...
9	18	4	0.6	3	0.5	3	0.5	..	...	..	...	..	...

It would seem that in this test for pancreatic function as originally devised, important variables have been overlooked. It is believed that at least the most serious ones have been pointed out and that a means has been suggested for overcoming them. This modification is offered with a certain amount of reservation, not because of the method itself but because of the factors entering into the reaction. While it is true that with these modifications it has been possible to obtain consistent results from day to day on the same patient even though the iodin absorption factor varied greatly, doubt as to the reliability of any starch-iodin reaction must still exist, because such a reaction must at best be an uncertain and changeable one in the presence of body fluids.

The bile from two gallbladders of cows obtained from freshly killed animals gave readings of 8 and 9 units by the Bassler technic, but failed to show any evidence of amylolytic activity by the modified technic when the iodin absorption factor was taken into consideration.

Aside from any question as to the value of the reaction itself, a far more important question remains to be settled; namely, whether or not testing duodenal juice for an amylolytic ferment is actually a test for pancreatic amylase. There is some doubt as to whether this amylolytic ferment, when found, has had its only source in the pancreas. Grutzner,[13] Mendeldorp[14] and Glaessner[15] have all conclusively demonstrated the presence of a diastatic ferment in the extract of duodenal gland substance of various animals. Pawlow[1] states that the juice secreted by the duodenal glands unquestionably contains a diastatic enzyme. Bergman, Dukes and Yarborough[16] found that of sixty-five intestinal extracts from five species of animals (ox, horse, pig, sheep and dog) only three failed to digest starch in neutral solution. Even extracts of submucosa of ileum and colon showed greater power of splitting starch than extracts of other regions of the intestines. They also were able to obtain powerful amylolytic action from extracts of skeletal muscle, mesenteric lymph glands, epidermic and dermal, and subcutaneous tissue in the pig. The apparently universal distribution of this amylolytic ferment makes the value of a test for pancreatic function based on this enzyme alone highly problematic.

The argument may be advanced that conclusions applied to man from experiments done on lower animals are unjustifiable. However, in one instance at least, it was possible to obtain a portion of duodenum from a patient examined at necropsy immediately after death. A glycerin extract of mucosa and one of submucosa was prepared by Dr. Fred Boerner who used the method described by Bergman, Dukes and Yarborough.[16] These extracts were tested for the presence of an amylolytic ferment. The method of Bassler was used, but instead of determining undigested starch by adding iodin at the end, each tube was tested for the presence of sugar. Definite reducing reactions with Benedict's solution were obtained and the tubes containing the largest amounts of extract gave the strongest reactions.

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13. Grutzner, P.: Arch. f. diges. Physiol. **12**:265, 1876.

14. Mendeldorp, cited by Luciana: Human Physiology, trans. by Welby, London, 1913, **2**:124.

15. Glaessner, Karl: Beitr. z. chem. Phys. u. Path. **1**:105, 1901-1902.

16. Bergman, H. D.; Dukes, H., and Yarborough, J. H.: A Study of the Enzymic Action of Extract of the Duodenal Gland Regions of Domestic Animals, J. Am. Vet. M. A. **18**:313 (June) 1924.

SOME METABOLIC ASPECTS OF CALCIUM THERAPY*

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It was shown by Marriott and Howland [1] that retention of phosphoric acid was an important factor in the acidosis which develops in nephritis. It was also pointed out by the same authors that although the acidosis could be overcome by administration of alkali, such as sodium bicarbonate, little else was accomplished. There was no reduction of serum phosphate. They also suggested the possibility of calcium therapy as a means of eliminating phosphates by the bowel.

More recent work on calcium therapy has been especially concerned with the problem of edema in nephrosis, and it appears [2] that calcium chlorid or other salts, such as ammonium chlorid, which leave a residue of mineral acid, will, by bringing the hydrogen ion concentration of the tissues nearer to their iso-electric points, cause the elimination of sodium and edematous fluid. However, the questions of phosphate elimination and other effects of calcium on mineral metabolism have not been so extensively investigated.

This paper deals, first, with the effect of various alkalis and also of calcium chlorid on phosphate elimination in urine and feces, and, second, with the effect of calcium acetate on mineral metabolism.

PROTOCOLS

EXPERIMENT 1.—*Effect of alkalis and of calcium chlorid on elimination of phosphorus in urine and feces.*

F. M., an instructor in the department of medicine, incidentally having a typical case of nephrosis with edema, restricted his diet on twelve consecutive days to measured amounts of prepared foodstuffs. During the fifth and sixth days he took in addition, in divided doses, a total of 60 cc. of a 25 per cent calcium acetate solution; during the seventh and eighth days he took similarly 60 cc. of a 25 per cent magnesium acetate solution; during the ninth and tenth days, 60 cc. of a 25 per cent potassium acetate solution, and during the last two days, 60 cc. of a 25 per cent calcium chlorid solution. Twenty-four hour specimens of urine and feces were collected for each of the last ten days of the experiment. The daily urines were terminated with the passing of the morning urine on arising; a single bowel evacuation occurred each day about an hour later. Total phosphorus was determined on each of the daily specimens, but the results (Table 1) are grouped into five periods of two days each.[3]

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* From the department of medicine, St. Louis University School of Medicine.

1. Marriott, W. M., and Howland, J.: Phosphate Retention, Arch. Int. Med. **18**:708 (Nov.) 1916.

2. Keith, N. M.; Barrier, C. W., and Wheelan, M.: Treatment of Nephritis and Edema with Calcium, J. A. M. A. **83**:666 (Aug. 30) 1924.

3. In both experiments excreta on the first two days were discarded as preliminary.

EXPERIMENT 2.—*Effect of calcium on mineral metabolism.*

A measured diet of known inorganic composition was taken by the subject (A. P. B.) on ten consecutive days. On each of the last four days a measured amount of calcium acetate solution was taken by mouth. Urines and feces were collected to represent the twenty-four hour periods and determinations made on each of the various inorganic elements. Results of these are given in Table 2.

The diet consisted of:

25 Gm. of peanut butter
25 Gm. of condensed milk (sweetened)
20 Gm. of deviled ham
140 Gm. of bananas
147 Gm. of graham crackers
20 Gm. of jelly
5 Gm. of prepared coffee (George Washington)
20 Gm. of sucrose
2,000 Gm. of tap water

The food value of this diet is approximately protein, 28 Gm.; fat, 36 Gm., and carbohydrate, 204 Gm., with a heat value of about 1,250 calories. Although less than maintenance, this diet satisfied the appetite during the first two days and was adhered to throughout the experiment. The inorganic composition of the diet as determined from analysis was: chlorin, 0.950 Gm.; sulphur, 0.3136 Gm.; phosphorus, 0.450 Gm.; sodium, 1.707 Gm.; potassium, 0.772 Gm.; calcium, 0.2937 Gm., and magnesium, 0.2626 Gm.

Collection of twenty-four hour urines was terminated with the morning urine passed on arising. In this experiment the urines were preserved with chloroform. The daily feces, as in the previous experiment, also consisted of a single specimen passed within a half hour after breakfast. This breakfast consisted only of four graham crackers, 20 Gm. of jelly, and coffee with sugar but not milk, and contained a relatively small amount of the daily inorganic intake.

On the last four days calcium acetate was taken as follows: 10 cc. of a 20 per cent solution a half hour before the noon meal; 10 cc. again a half hour before the evening meal, and 5 cc. at bedtime. The amount of calcium taken daily in this way was approximately 1.14 Gm.

METHODS OF ANALYSIS

Urines were measured, diluted with distilled water to 1,500 cc., and mixed. Feces were passed through a sieve and diluted to 500 cc. and mixed. Measured or weighed samples of urine, feces and food were dried and charred in a platinum dish, the charred mass extracted with water, and the residue ignited. This ash was dissolved in dilute hydrochloric acid, added to the water extract, and the whole evaporated to a convenient volume, from which aliquots were taken for the determinations of sodium, potassium, calcium, magnesium and phosphorus. Chlorin and sulphur were determined on separate samples. Calcium, magnesium, potassium and phosphorus were determined essentially as reported in a previous paper;[4] however, in this study the samples taken for analysis contained approximately ten times as much of the inorganic element concerned as in the previous work which was done on blood plasma. Sodium was determined by the volumetric method of Kramer.[5]

4. Briggs, A. P.: J. Biol. Chem. **57**:351 (Sept.) 1923.
5. Kramer, B., and Gittleman, I.: J. Biol. Chem. **62**:353, 1924.

Chlorin was done by the wet ash method of Van Slyke,[6] and sulphur by the method of Benedict.[7]

It is commonly stated [8] that alkali causes more phosphates to be eliminated in the feces and less in the urine. In their study on the mineral metabolism of infants Shohl and Sato [9] found a slight change in that direction with the administration of sodium bicarbonate. The

TABLE 1.—*Excretion of Phosphorus During Two-Day Periods on Various Salts (Milligrams Phosphorus)*

	Period				
	Control	Calcium Acetate Solution	Magnesium Acetate Solution	Potassium Acetate Solution	Calcium Chlorid Solution
Urine phosphorus	976	494	935	905	690
Feces phosphorus	623	810	585	625	650
Total	1,599	1,304	1,510	1,530	1,340

TABLE 2.—*Minerals Excreted in Urine and Feces (Milligrams per Twenty-four Hours) in Experiment 2*

	Sodium	Potassium	Calcium	Magnesium	Chlorin	Phosphorus	Sulphur
Urine control period	2,990	870	241	149	1,320	484	520
	1,900	1,120	189	168	1,020	484	400
	1,900	1,220	147	152	1,020	455	394
	1,600	1,420	126	156	790	470	206
Total	8,390	4,630	703	625	4,150	1,893	1,520
Urine calcium acetate period	1,700	1,120	315	196	945	395	437
	2,300	1,280	294	173	836	234	350
	1,400	1,280	284	156	826	250	330
	1,300	1,280	294	180	915	220	384
Total	5,700	4,960	1,187	705	3,512	1,099	1,501
Feces control period	370	180	415	360	20	470	150
	130	100	161	68	16	117	111
	130	110	77	75	11	154	148
	230	180	168	78	11	263	63
Total	860	570	821	690	58	1,004	472
Feces calcium acetate period	200	100	455	123	12	360	82
	400	180	1,245	246	15	625	143
	400	123	980	151	12	360	76
	370	88	1,110	109	16	264	96
Total	1,370	491	3,790	629	55	1,609	397

results shown in Table 1 indicate that potassium or magnesium acetate have little influence on the partition of phosphorus, whereas either calcium acetate or calcium chlorid lead to the diversion of considerable amounts of phosphorus from the urine to the feces. This brings to mind the experiments of Fiske and Sokhey [10] in which the administra-

6. Van Slyke, D. D.: J. Biol. Chem. **58**:523 (Dec.) 1923.
7. Benedict, S. R.: J. Biol. Chem. **6**:363, 1909.
8. Shohl, A. T.: Physiological Rev. **3**:529 (Oct.) 1923.
9. Shohl, A. T., and Sato, A.: J. Biol. Chem. **58**:248 (Nov.) 1923.
10. Fiske, C. H., and Sokhey, S. S.: J. Biol. Chem. **63**:309 (March) 1925.

tion of small amounts of mineral acid by various pathways led to no change in phosphate excretion.

Values for the minerals excreted in the second experiment are given in Table 2. Balances for each are shown in Table 3. Since the diet in this experiment was below maintenance, the deficiency was probably met at the expense of skeletal muscle.[11] This probably accounts for the negative balances of potassium, magnesium, phosphorus and sulphur.

Sodium and chlorin were in approximate equilibrium during the greater part of the experiment.

TABLE 3.—*Balance of the Various Minerals in Experiment 2*

	Sodium	Potassium	Calcium	Magnesium	Chlorin	Phosphorus	Sulphur
Control Period							
Urine.....	8,390	4,630	703	623	4,150	1,893	1,520
Feces.....	860	570	821	600	58	1,004	472
Total.....	9,250	5,200	524	1,314	4,208	2,897	1,992
Intake.....	6,828	3,088	1,175	1,040	3,790	1,800	1,254
Balance.....	−2,422	−2,112	−349	−274	−418	−1,097	−738
Calcium Acetate Period							
Urine.....	5,700	4,960	1,187	705	3,512	1,099	1,501
Feces.....	1,370	491	3,790	629	55	1,609	397
Total.....	7,070	5,451	4,977	1,334	3,567	2,708	1,898
Intake.....	6,828	3,088	5,715	1,040	3,790	1,800	1,254
Balance.....	−242	−2,363	+738	−294	+223	−908	−644

TABLE 4.—*Daily Excretion of Ammonia in Experiment 2*

	Day							
	3	4	5	6	7*	8	9	10
Cubic centimeters of tenth normal ammonium hydroxid	190	173	173	173	121	104	104	86

* Calcium acetate was taken on the last four days.

With the ingestion of calcium acetate, the small negative calcium balance was changed to a positive one. Presumably depleted calcium reserves were being restored at this time, and the coincident decrease in the negative phosphorus balance indicates the storage of some calcium phosphate.

In spite of a definite decrease in total phosphorus elimination during the calcium acetate period, there is a conspicuous increase in the phosphorus excreted with the feces. In a clinical report to follow it will be shown that the elevated plasma phosphate in nephritis can be brought down to normal by the administration of calcium acetate.

The excretion of sodium potassium and magnesium is not appreciably influenced by calcium acetate, which, together with other recent

11. Gamble, J. L.; Ross, G. S., and Tisdall, F. F.: J. Biol. Chem. **57**:640 (Oct.) 1923.

work,[12] supports the theory that the increased sodium elimination following the administration of calcium chlorid is due, not to an antagonism between cations, but to the residue of hydrochloric acid from excretion of calcium by the bowel.

As previously stated, it seems probable that the positive balance for calcium with the administration of calcium acetate is due to the storage of calcium carbonate and phosphate in solid tissues. Certainly there appears to be nothing brought out in this study to suggest that administration of calcium acetate to a nephritic patient would lead to retention of alkali with edema.

Whether various other salts are excreted in the urine passively or not, the ammonia appears to be synthesized by the kidney [13] as an important agent in neutrality regulation and base conservation; [11] hence the increase of ammonia found in the urine following the administration of calcium chlorid which leaves a residue of hydrochloric acid. On the other hand, the administration of an organic salt of calcium, such as calcium acetate, should tend to decrease the formation of ammonia, since the organic radicle would be oxidized and eliminated by the lungs and the calcium component would divert a part of the phosphoric acid from the urine to the feces. Ammonia was determined in the urines in Experiment 2. The results shown in Table 4 support the contention that a certain amount of the phosphoric acid load has been taken by the intestine from the kidney.

SUMMARY

Calcium acetate given by mouth causes the diversion of a considerable amount of the excreted phosphoric acid from the urine to the feces.

The relief from this phosphoric acid load necessitates the synthesis of a smaller amount of ammonia by the kidney.

The administration of calcium acetate affords a means of correcting the phosphoric acid retention in nephritis free from the disadvantages of alkali retention and resulting edema which is observed with sodium bicarbonate therapy.

12. Keith (Footnote 2). Atchley, D. W.; Loeb, R. F., and Benedict, E. M.: Physicochemical Studies of Calcium Chlorid Diuresis, J. A. M. A. **80**:1643 (June 2) 1923.

13. Nash, T. P., and Benedict, S. R.: J. Biol. Chem. **48**:463 (Oct.) 1921.

EFFECTS OF INTRAVENOUS INJECTIONS OF ACRIFLAVINE IN SEPSIS *

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The last few years have witnessed the trial of several antiseptic dyes, intravenously, in sepsis and bacteremia. Of these, neutral acriflavine, gentian violet and mercurochrome have been reported favorably.

Acriflavine, of which the chemical formula is 3-6 diamino-10 methylacridinium dihydrochlorid, was introduced by Ehrlich, who found it effective against trypanosomes and who called it trypaflavine. Browning,[1] who had worked with Ehrlich, together with his co-workers, found that the dye was antiseptic, that it maintained its efficiency even in serum, and that a solution of 1 : 100,000 killed staphylococci and *Bacillus coli*. They also advocated the use of the dye in war wounds as a dressing after proper surgical measures had been taken. The dye was used intensively by a number of the British surgeons, but not all would subscribe to its value. Ligat [2] found it useful, but most [3] of them emphasized the early use of débridement and the relative inertness of the dye in later stages of infected wounds. In arguments against Browning's data of the potency of acriflavine, Fleming [4] administered the dye intravenously to a rabbit and then found the blood to have no effect on staphylococci. Hewlett [5] also found the dye much weaker than Browning stated.

Because of its penetrating power the drug was used locally in the treatment of gonorrhea by a large number of observers with contradictory results.[6] Finally, it was used intravenously for the treatment

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* From the medical service of the Fifth Avenue Hospital.

1. Browning, C. H.; Gulbranson, R.; Kennaway, E. L., and Thornton, L. H. D.: Flavine and Brilliant Green, Brit. M. J. **1**:73 (Jan. 20) 1917. Browning, C. H.; Gulbranson, R., and Thornton, L. H. D.: Antiseptic Properties of Acriflavine and Proflavine, Brit. M. J. **2**:70 (July 21) 1917.

2. Ligat, D.: Flavine and Brilliant Green in the Treatment of Infected Wounds, Brit. M. J. **1**:78 (Jan. 20) 1917.

3. Drummond, H.; Hamilton and McNee, J. W.: Treatment of a Series of Recently Infected War Wounds with Flavine, Lancet **2**:641 (Oct. 27) 1917. Colledge, L.; Drummond, H.; Worthington, R. T.; McNee, J. W.; Sladden, A. F., and McCartney, J. E.: Treatment of a Series of Recently Infected War Wounds with Proflavine, Lancet **2**:676 (Nov. 3) 1917.

4. Fleming, A.: Physiological and Antiseptic Action of Flavine, Lancet **2**:340 (Sept. 1) 1917.

5. Hewlett, R. T.: Germicidal Power of Flavine, Lancet **2**:621, 1917.

6. Hyman, Abraham: Acriflavine in the Treatment of Gonorrhea, Urol. & Cutan. Rev. **24**:325, 1920.

of puerperal and other infections. Bohland [7] reported its use in ten cases of influenza and pneumonia, in which he obtained great improvement with lysis. One other patient, however, developed meningism and empyema, despite the treatment, before recovery. He cites another case, one of pyelonephritis with cystitis with recovery, and also three cases of puerperal sepsis. Bohland used a solution of 0.5 per cent. and gave 0.1 gm. daily. He recommended the drug in all cases of sepsis.[8] Cramer,[9] in ten cases out of eleven of puerperal fever, got good results, but in one case of sepsis following tonsillar abscess, death followed. Whitehouse [10] used a solution of 1 : 1,000 intravenously in doses of from 10 to 15 c.c. daily and was pleased with the results, but he reserved his judgment of its value. Spiess,[11] in a case of postoperative meningitis following transnasal removal of an hypophyseal tumor, revised and drained the operative site and gave trypaflavin intravenously, with recovery by the patient.

We have administered neutral acriflavine by the intravenous route, using the commercial preparation especially purified for that purpose, in eleven cases of generalized infection. Of these, six had a positive blood culture, and it is to these that we wish to call attention, since these cases, we consider, offer a direct criterion of the efficacy of the drug. In each case a 1 per cent. solution was freshly prepared with physiologic sodium chlorid solution made that same day from freshly distilled water, then filtered and sterilized by boiling for twenty minutes. The average dose was 5 mg. of the dye per kilogram of body weight, at intervals stated in the protocols. In general, the injections were well tolerated, the only untoward symptom being nausea and, rarely, vomiting in one or two of the patients. No hematuria or suppression or other evidence of damage in renal function was noted in these patients, such as found by Bohland [7] in a nephritic patient or by Meleney and Zau [12] in experimental work on rabbits.

REPORT OF CASES

CASE 1.—*Carbuncle of lip; lobar pneumonia; septicemia (Staphylococcus aureus).* R. P., a man, aged 30, had an infection of the upper lip of twenty-four hours' duration, which had started from a small fever blister, and was accom-

7. Bohland, K.: Intravenöse Anwendung des Trypaflavins bei Infektionskrankheiten, Deutsch. med. Wchnschr. **45**:797, 1919.

8. Bohland, K.: Trypaflavine, ein inneres Antisepticum, Med. Klin. **15**:1173, 1919.

9. Cramer: Erfahrungen mit intravenoser Injektion des Trypaflavins bei puerperalen Infektionen, Deutsch. med. Wchnschr. **46**:311, 1920.

10. Whitehouse, H. B.: Surgical Treatment of Puerperal Sepsis, Brit. M. J. **2**:267 (Aug. 21) 1920.

11. Spiess, G.: Heilung eines Falles von Meningitis nach endonasaler Operation eines Hypophysentumors durch Trypaflavininfusionen, Deutsch. med. Wchnschr. **46**:207 (Feb. 19) 1920.

12. Meleney, F. L., and Zau, Z.: Action of Acriflavine in the Blood and Certain Tissues of Rabbits, J. A. M. A. **84**:337 (Jan. 31) 1925.

panied by chill, fever and abdominal rigidity. The patient was prostrated, showing marked swelling of the upper lip with pus oozing and signs of consolidation of the right lower lobe. The white cells totaled 12,000, of which the polymorphonuclears were 94 per cent. and the lymphocytes 6 per cent. A blood culture showed *Staphylococcus aureus*, and a culture from the lip showed *Staphylococcus aureus*. The temperature ranged between 102.2 and 106 degrees.

Two injections of neutral acriflavine solution were given. The patient died two days after admission.

Case 2.—*Endocarditis acuta.* A. D., a woman, aged 44, with a history of measles, and of rheumatism at 17 and again at 41, was admitted complaining of fatigue and weakness for one month and shortness of breath for two weeks. She had a slight cough and occasional night sweats. The heart was found enlarged to the left, with a blowing murmur. There were moist râles over the posterior chest. The spleen was enlarged. The urine showed a faint trace of albumin, with occasional hyaline casts. Blood examination showed: hemoglobin, 89 per cent.; red cells, 4,000,000; white cells, 10,000, with 80 per cent. polymorphonuclears. A blood culture showed *Streptococcus viridans*.

Beginning the third day in the hospital, acriflavine was given daily for eight doses. The temperature ranged between 102 and 97 degrees in a septic manner. Orthopnea and edema followed, with precordial distress. The patient died on the twelfth day in the hospital.

Case 3.—*Endocarditis acuta; Streptococcus viridans bacteremia.* E. R., a woman, aged 25, had a history of scarlet fever at 8, followed by rheumatism and heart trouble. At 14 she had rheumatism again and heart trouble. Sixteen days before admission she developed pains in the wrists, hands, chest and back. The pains involved all the joints for ten days, and stopped only to recur later together with vomiting. She was undernourished and irrational. Opisthotonos was present. The great toe phenomenon of Babinski was positive on the right and Kernig's sign was present. The urine had a heavy trace of albumin with red cells, pus and few casts. The heart was enlarged to the left, and a loud systolic blow was present over the precordium. A blood culture showed *Streptococcus viridans*, but the spinal fluid was sterile though bloody.

Neutral acriflavine was given intravenously every second day for five doses; then after a free period of ten days it was given daily for four doses. The temperature ranged between 97 and 106 degrees. Blood cultures remained positive. The hemoglobin, at first 81 per cent., fell to 47 per cent.; the red cells from 3,800,000 to 2,500,000. The patient died three months after admission.

Case 4.—*Endocarditis subacuta; Streptococcus viridans bacteremia.* G. M., a man, aged 23, had acute rheumatic fever at the age of 5 which kept him ill for two years and left him with impaired cardiac reserve. For three months before admission he had aches and pains of moderate severity, fever and a "cold" (possibly tonsillitis), and had to stay in bed. He was pale; the tonsils were large and red. The heart was enlarged, with a to-and-fro murmur at the apex and a diastolic murmur at the base. The liver and spleen were enlarged. The fingers were clubbed. Hemoglobin was 79 per cent.; red cells totaled 4,000,000; white cells, 16,500, with polymorphonuclears 76 per cent. and lymphocytes 24 per cent. The blood showed *Streptococcus viridans*.

The patient was given eleven injections of neutral acriflavine in a period of twelve days. These caused general discomfort, nausea and vomiting immediately afterward, and his skin, conjunctivae, urine and feces were all deep yellow. The blood culture, however, was persistently positive. He was given thirteen daily injections of sodium cacodylate, with no improvement. The hemoglobin gradually dropped to 53 per cent., the red cells to 2,700,000. He was twice transfused with whole blood by Dr. S. A. Thompson. The patient died seven weeks after admission.

Case 5.—Typhoid fever. D. Z., a man, aged 24, was admitted complaining of malaise for five days and a chill four days before, followed by fever and sweats. The only positive physical findings were a congested pharynx and a

palpable spleen. The white cells were 5,500, with polymorphonuclears 64 per cent., lymphocytes 26 per cent., transitionals 9 per cent. and eosinophils 1 per cent. The Widal agglutination test of the serum was negative for typhoid and paratyphoid, but the blood culture was positive for *Bacillus typhosus.* The patient was only moderately sick.

He was given five daily injections of neutral acriflavine, beginning three days after admission. The temperature ranged between 102 and 104 degrees for twelve days, then gradually fell to normal. No effects were seen from the acriflavine, and it was forty days after admission before negative stools were obtained and the patient relieved from quarantine.

CASE 6.—*Sepsis; Streptococcus hemolyticus; complicating diabetes.* J. A., a man, aged 48, had been under treatment for diabetes for more than a year. He developed thrombophlebitis of one leg, with fever, prostration and coma. A blood culture showed *Streptococcus hemolyticus.*

He was given four injections of neutral acriflavine at intervals of eight hours. There was no change and the patient died.

Two of these cases were fulminating infections, one patient with *Streptococcus hemolyticus* dying within twenty-four hours from the time of initial observation and treatment, the other, with *Staphylococcus aureus,* dying in thirty-six hours from the onset. One case of mild typhoid passed through the usual course even without apparent shortening of the period of infectivity. Three cases of endocarditis with *Streptococcus viridans* bacteremia, with from nine to eleven injections of neutral acriflavine, ran their course unaltered, with no change observed in the positive blood culture at any time during or after the injection.

In this connection it is of interest to review the work of Spencer.[13] He injected rabbits and fifteen minutes later tested the bactericidal effect of the blood on *Bacillus typhosus* in vitro, according to the looped pipet method of Wright. A single injection of 1 c.c. of dye in a concentration of 1 : 100 caused no difference fifteen minutes afterward. The test was repeated with *Staphylococcus aureus,* and repeated injections tried for cumulative effect. Thus, after four injections at twenty minute intervals with the maximal tolerated dose, no change was found in the bactericidal power of the blood. Similarly, on injecting white mice with the minimal lethal dose of pneumococcus culture followed by the maximal tolerated dose of acriflavine, none was saved. Likewise, Meleney and Zau [12] found that when the dye was given intravenously to rabbits even in lethal doses, there were no bactericidal or bacteristatic properties for *Streptococcus hemolyticus.* When a minimal lethal dose of washed hemolytic streptococcus (which would kill a control in from seven to ten days) was injected intravenously and treatment with acriflavine was instituted from twenty-four to forty-eight hours afterward, the dye did not prevent death but actually hastened it. They found that with

13. Spencer, Harvey: Effects of the Intravenous Injections of Acriflavine, J. Lab. & Clin. Med. **9**:322 (Feb.) 1924.

lethal doses marked kidney damage was done and that with nonlethal doses there was a rise in blood urea during the first few days. They therefore concluded that there was no sterilizing action within the body of the rabbit and that the injury the dye produced in the liver and kidneys might even handicap the animal in its fight against the infection.

That many patients with severe infections who have received acriflavine have recovered is certainly undeniable. But on the other hand, in other cases of equal severity there has been recovery without such treatment. Spontaneous recovery in *Bacillus coli* sepsis was reported by Jacob [14] in 60 per cent. of thirty-nine cases. Coleman and Hastings [15] reported three such cases, and recently Felty and Keefer [16] have reported seventeen recoveries in a series of twenty-five cases (58 per cent.). Likewise, spontaneous recovery occurs for other organisms. Thus, speaking of the serum treatment, Kolmer [17] states: "The tendency of acute streptococcus infections to end spontaneously by crisis must, however, be borne in mind, and the good results observed in individual cases may be coincident with, rather than the result of, the administration of serum."

We record two cases of *Streptococcus hemolyticus* sepsis in which there was recovery without any noteworthy treatment.

CASE 7.—*Metritis; Streptococcus hemolyticus septicemia; recovery.* L. M., a girl, aged 18, a negress, was delivered normally of a healthy child two weeks before admission to the hospital. She was discharged well one week after delivery, and that night began to have severe, cramplike pain in the lower abdomen and in the side. The pains continued constantly for six days, but eased up on the morning of admission to the hospital. She had had complete anorexia, marked thirst and high fever. She was acutely ill, prostrate and very tender all over the abdomen, with rigid abdominal muscles. The uterus was enlarged almost to the umbilicus. The head and thorax were normal. The temperature was 103; the pulse, 120, and respiration, 36. The white cells totaled 22,400, with 73 per cent. polymorphonuclears. A blood culture showed *Streptococcus hemolyticus.* The urine showed a trace of albumin, with occasional hyaline casts and white cells, both scattered and clumped.

The uterus was curetted forty-eight hours after admission and a small amount of secundines removed. Hot douches of a proprietary solution of an antiseptic preparation were given three times a day. The temperature varied between 103.8 and 98.4 for seven days, then stayed normal. Blood cultures eleven days after admission were sterile. The patient made a complete recovery.

CASE 8.—*Streptococcus hemolyticus septicemia, secondary to otitis media and venous thrombosis; recovery.* J. H. K., a boy, aged 15, had had scarlet fever eight years before complicated by right otitis media. The following year the

14. Jacob, L.: Colon Bacillus Septicemia, Deutsch. Arch. f. klin. Med. **97**:303, 1909.

15. Coleman, W., and Hastings, T. W.: Bacillus Coli Communis: The Cause of an Infection Clincally Identical with Typhoid Fever, Am. J. M. Sc. **137**:199, 1909.

16. Felty, A .R., and Keefer, C. S.: Bacillus Coli Sepsis: Clinical Study of Twenty-Eight Cases of Blood Stream Infection by the Colon Bacillus, J. A. M. A. **82**:1430 (May 3) 1924.

17. Kolmer, J. A.: Infection, Immunity and Biologic Therapy, Ed. 3, Philadelphia, W. B. Saunders Company, 1924, p. 893.

tonsils and adenoids were removed because of frequent sore throats and nose colds. He was well until one month before admission, when he developed bilateral earache, which kept him in the school infirmary for three days. Two weeks before admission he had frequent sensations of chill and a sore throat. Three days later the left ear ached, and five days later a myringotomy was done, with relief of only a few hours. Then he began to have a generalized headache which persisted although the left ear continued to drain pus for five days. He had sensations of chill and weakness afterward, and on the morning of admission he vomited. The temperature for the three days preceding ranged between 106 and 100.

The physical examination was entirely normal except for a slight serous discharge from the left ear. The eye-grounds were repeatedly examined and found normal. The white cells totaled 18,000, with 75 per cent. polymorphonuclears. The temperature on admission was 100.4, then dropped to 98.6. A chill followed, and the temperature rose to 106. This dropped within twelve hours to 96, rose to 103, and gradually fell in the next two days to normal. A blood culture was positive for *Streptococcus hemolyticus* on admission, and again on the second and fourth days. One week after admission the blood culture was sterile. The boy made a complete recovery.

CONCLUSIONS

Of eleven patients with generalized infection who were treated with intravenous injections of neutral acriflavine, six had positive blood cultures. Of these six, five died despite the treatment. The sixth case, one of typhoid fever, ran the usual course and showed no shortening of the time necessary to procure negative stool cultures for release from quarantine.

We have seen no improvement following the intravenous use of neutral acriflavine in cases of sepsis or bacteremia.

There was spontaneous recovery in two cases of *Streptococcus hemolyticus* sepsis.

Book Reviews

MODERN MEDICINE, ITS THEORY AND PRACTICE IN ORIGINAL CONTRIBUTIONS BY AMERICAN AND FOREIGN AUTHORS. Edited by Sir William Osler. Third edition, thoroughly revised, reedited by Thomas McCrae, assisted by Elmer H. Funk. Vol. 1. Illustrated. Philadelphia: Lea and Febiger, 1925.

The first volume of the third edition of Osler's Modern Medicine, revised and enlarged, contains many features which stamp it as a valuable "bread and butter" volume for the general practitioner. The aim of the editor has been to "keep an even balance between the condensation of a textbook and the elaborate treatment of the monograph." The volume contains Osler's original introduction of the first edition on the Evolution of Internal Medicine, an interesting chapter from an historical standpoint which contains much useful but not strictly medical information; it is valuable chiefly because it reflects Osler's point of view, as Osler himself lived through the period in which internal medicine as such evolved into a strict specialty.

Many of the original contributors have died: Sir William Osler, John H. Musser, James Carroll, W. P. Dunbar, Isadore Dyer, John H. McCollum, M. Herzog, A. O. J. Kelly, John McCrae, and E. H. Southard. In some instances their names have been retained at the head of the articles originally contributed by them but which have been revised; this is true of all of Osler's articles. This volume, on infectious diseases, contains chapters on the Introduction to the Study of Infectious Diseases, by Ludvig Hektoen; Typhoid Fever, by Thomas McCrae; Lobar Pneumonia, by George William Norris and David L. Finley; Tuberculosis, by Edward R. Baldwin; Diphtheria and Scarlet Fever, by John H. McCollum and Edwin H. Place, revised and brought up to date by Thomas McCrae and Elmer H. Funk. This series promises to meet the requirements of the general practitioner, in providing an extremely helpful and usable reference work, and will undoubtedly meet with universal approval.

SYPHILIS UND INNERE MEDIZIN: I TEIL, DIE ARTHRO-LUES TARDIVA UND IHRE THERAPIE. By HERMANN SCHLESINGER. Pp. 165; 8 illustrations. Price, 9.90 gold marks. Vienna: Julius Springer, 1925.

This well written monograph concerning arthrosyphilis tardiva is the first of a series devoted to syphilis and internal medicine. The two parts in preparation will deal with the relation of syphilis to other internal diseases and to the glands of internal secretion.

The present work, based on a study of thirty-five cases, describes the hitherto largely neglected forms of joint conditions due to syphilis in which other signs of syphilis are absent. The Wassermann reaction of the blood is of no value in diagnosis since it usually is negative and frequently remains negative throughout treatment. A positive reaction of the joint fluid, found in some cases, is of diagnostic significance. Luetin tests, also, are of no value. The writer believes that, exclusive of cases of septic origin, gonorrhea and late syphilis are the most frequent causes of the acute joint affections refractory to salicylates. Late syphilis is also responsible for a fairly large number of the varied group of chronic arthritides. The true nature of these cases is apt to be overlooked unless, particularly in every chronic joint affection aside from those of gouty, gonorrheal or traumatic origin, the possibility of syphilitic etiology is kept in mind.

The clinical classification offered includes arthralgias, acute or subacute febrile forms, chronic cases, atypical types and mixed forms. Tabetic arthrop-

athies are not considered as forms of arthrosyphilis. Spinal localizations are discussed under the headings of spondylitis syphilitica simplex and destructiva. The roentgen-ray findings are fully described. The criteria for diagnosis are tabulated and carefully evaluated and differential diagnosis considered. Pathologic anatomy receives attention. An extensive bibliography and an index are included.

This careful clinical study, directing attention to a group of joint conditions likely to be neglected as to diagnosis and treatment, is of practical importance and deserves wide reading. Its presentation is clear and concise.

TEXT BOOK OF PATHOLOGY. Thirteenth Edition. By F. DELAFIELD and T. M. PRUDDEN. Price, $10. New York: William Wood & Co.

The thirteenth edition of Delafield and Prudden's well known Text Book of Pathology has appeared. On the whole, the form and arrangement of the contents have not been materially altered either in this edition or in recent former editions.

Attention is called in the preface to the large amount of chemical and physiologic data that is of interest in the field of pathology, much of which has not as yet been correlated or digested. To do this will take time. Authors of pathology textbooks and especially those concerned in the preparation of revisions will find a fertile field for many years to come in selecting and sifting contributions in both near and remote fields and arranging them in such a way that they will become satisfactorily incorporated in standard works. The tendency to multiply texts in various special or related fields will not solve the problem. The time allotted for courses in the medical curriculum is so limited that textbooks of wide scope but with condensed contents will always be necessary for medical students.

In this edition of Delafield and Prudden, the impression may be obtained that undue conservatism exists in the consideration of recent important contributions. For example, no mention is made of the work of Noguchi on yellow fever and the discovery of the *Leptospira icteroides*. So, too, though mention is made of the revision of the work on lung abscess and gangrene, the numerous contributions and the real advances that have been made in connection with the rôle of spirochetes in these processes are not set forth. It would seem, too, that a clearer statement than is given is now possible concerning the pathogenicity of the so-called influenza bacillus and its nonrelationship to epidemic influenza, in view of the numerous researches that have been completed since the last influenza pandemic.

On the whole, however, the more important contributions in the field have been reviewed or are mentioned in footnotes together with references that cover a wide range of literature. The book continues to be one of the most useful texts in its field and in many ways is better adapted for the teaching of pathology, as the subject has been developed in America, than any other.